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EARLY GASTRIC CANCER*

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ROCHESTER, MINN.

Hauser,[1] in 1883, was the first to note histologic evidence of the fact that gastric cancer may arise in chronic gastric ulcer, and since that time there have been numerous and varying contributions to the literature on this subject. Today, with the newer methods and advances in gastric surgery, earlier and smaller lesions may be studied. The examination of fresh unfixed material gives a more accurate cytologic and histologic picture, thereby reducing the number of borderline diagnoses and permitting the earlier recognition of malignant changes. In the past, a diagnosis of cancer of the stomach was not made until all the classic signs and symptoms of a malignant tumor were present. In this stage, treatment is at best only palliative, although certain patients may live for years after resection. In general, the postoperative good results are indirectly proportional to the size of the growth. Therefore, it behooves the medical profession to attempt all means which allow the recognition of the smallest malignant condition.

MATERIAL

In this study 100 excised and resected small gastric lesions were used. The gross appearance of each lesion was noted. Fresh frozen sections were made from various portions and stained with Unna's polychrome methylene-blue ripened according to Terry's rapid method. Fixed frozen sections stained with hematoxylin and eosin were also studied.

OBSERVATIONS IN CHRONIC GASTRIC ULCER

The chronic gastric ulcer is a local circumscribed dissolution of the continuity of the gastric wall involving all layers, and is usually round, funnel-shaped or U-shaped. The size varies from a few millimeters to several centimeters in diameter. In the mucosa of the borders of such ulcers, the capillaries are congested and there is an increase in the interstitial tissue, or fixed connective tissue, with lymphocytic infiltration. The tubules are usually tortuous, and some, especially those nearest the

* Submitted for publication, Sept. 5, 1929.

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1. Hauser, Gustav: The Chronic Gastric Ulcer, Its Cicatrization, and the Relationship Between the Development of Gastric Carcinoma, Leipzig, F. C. W. Vogel, 1883.

muscularis mucosae, are sometimes dilated. This torsion and budding of the tubules give the histologic appearance of glandular hyperplasia. The epithelium that lines the tubules usually shows some regeneration, and occasionally there may be seen one or more heterotropic glands beneath the muscularis mucosae. The muscularis mucosae is often thickened and blends with the muscularis and scar tissue in the crater. The submucosa is also thickened and infiltrated with inflammatory fibrous tissue. The muscularis in the base of the ulcer is almost completely replaced by fibrous tissue; it is hyalinized in older cases, and it is tilted upward toward the border of the ulcer, blending with the muscularis mucosae. This traction of the muscular coats, together with the scar tissue in the crater, often causes overhanging of the mucosa. The subserosa is usually thickened by fibrous tissue infiltration. In the upper portion of the concave base may be observed degenerated mucus, cell débris, degenerated epithelium, bacteria, small round cells, polymorphonuclear leukocytes, plasma cells, fibroblasts and fibrocytes. The vascularity of the crater varies. In some cases it presents numerous small capillaries; in others, fewer larger well formed capillaries, and in still others few capillaries or blood spaces. Throughout, and in any part of the ulcer or in its edges, in mucosa, crater, musculature or subserosa, there may be aggregations of lymphocytes, sometimes forming definite groups with germinal centers. The blood vessels supplying the ulcer are frequently thickened.

The causes of gastric ulcer are not known. Injury caused by or associated with arterial spasm, hemorrhage in local tissue or embolic infarcts may play a large part. Portal stasis with variations in local blood pressure may possibly produce such hemorrhage with necrosis. Chemical and thermal trauma may be causative factors.[2] Rosenow[3] produced acute and subacute ulcers in animals by injection of bacteria obtained from foci of infection in cases of peptic ulcer. Mann[4] produced similar ulcers, however, in other ways. Experimentally produced ulcers have not been observed becoming chronic and remaining so; for the occurrence of chronicity there must be interference with local circulation. This may be the reason why chronic gastric ulcers have never been produced experimentally.

Vascular change probably plays an important part in preventing ulcers from healing. The more intense the acute and subacute inflammatory reaction the more rapid the healing process. If the inflammatory

2. Aschoff, L.: Ueber die mechanischen Momente in der Pathogenese des runden Magenschwürs und über seine Beziehungen zum Krebs, Deutsche med. Wchnschr. **38**:494, 1912.

3. Rosenow, E. C.: The Causation of Gastric and Duodenal Ulcer by Streptococci, J. Infect. Dis. **19**:333, 1916.

4. Mann, F. C.: Production and Healing of Peptic Ulcer, Minnesota Med. **8**:638, 1925.

process is diminished or entirely absent, the alkalinizing influence of the exuding serum of the capillary bed in the crater neutralizing the local action of the gastric juice will not be apparent. In such a case connective tissue will not be formed as a result of inflammatory reaction, and the ulcer will affect the deeper structures until the serosa is reached and perforation occurs. There is a constant struggle between the healing and the ulcerative forces.

The mucosa lining the stomach contains relatively simple tubular glands.[5] There are three or four different kinds of cells lining these tubules with quantitative variations in different portions of the stomach. The first type of cell is the surface secreting cell, which includes the cells lining the surface of the ducts leading from the deeper portions of the glands. The second is the mucoid cell, of which there are two closely allied groups, namely, the cardiac and pyloric glands and the mucoid cells that occur in the large intervening fundus, where they are intermingled with the peptic and oxyntic cells; they occupy chiefly the superficial or upper half of the gland tubule, but may occasionally be seen almost throughout the tubule. The oxyntic cells, which chiefly occupy the upper portion of the gland, are found between the mucoid cells; in the deeper portion of the gland, they take up a parietal position. The peptic cells are found within the deeper part of the gland. From the study of new-born cats it is found that the peptic cells arise from cells of the mucoid type and are the last to develop. The mucoid cell is a stage in the genesis of the peptic cell. The peptic cells and the oxyntic cells are the most highly differentiated and specialized.

It would be difficult to prove that a benign gastric ulcer may change into a carcinomatous or malignant ulcer unless one could produce experimentally chronic gastric ulcer, then produce cancer in the ulcer, and then show that all the conditions of the experiments are comparable with the conditions that arise in human beings.[6] Hauser attempted in a rather convincing manner to demonstrate histologically that the change does occur; he distinguished atypical tubular proliferation from true cancer.

Friedlander[7] concluded from his investigations that atypical epithelial hyperplasia may occur where regenerative processes are taking place, either in the epithelium-bearing membrane itself or in the surrounding structures. These processes are either producing granulation tissue or specific tumor. Von Hansemann[8] believed that the epithelium

5. Lim, R. K.: The Gastric Mucosa, Quart. J. Micr. Sc. **66**:187, 1922.

6. MacCarty, W. C.: Chronic Ulcer and Carcinoma of the Stomach, Am. J. M. Sc. **173**:466, 1927.

7. Friedlander, quoted by Hauser (footnote 1).

8. Von Hansemann, D.: Ueber die Funktion der Geschwulstzellen, Ztschr. f. Krebsforsch. **4**:564, 1906.

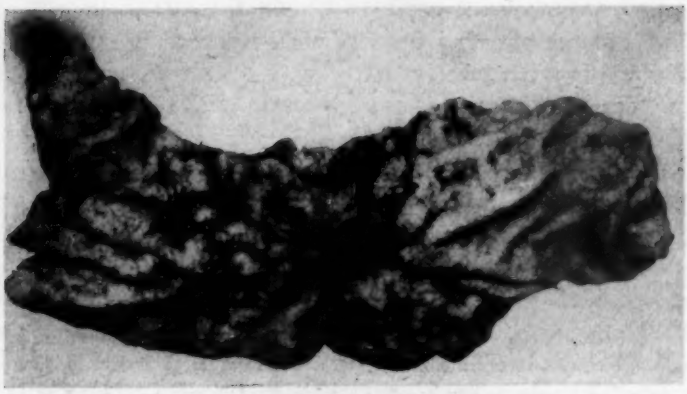

Fig. 1.—Simple chronic gastric ulcer (1.5 by 1.5 by 1 cm.).

Fig. 2.—Chronic gastric ulcer (1 cm. in diameter) with cancer in mucosa.

Fig. 3.—Chronic gastric ulcer (6 by 4 by 2 cm.) with cancer in mucosa.

returns to its embryonic state and acquires a significant reproductive property which belongs to undifferentiated tissue. If at this time it is excited to proliferate, it produces new and atypical tissue known as cancer. MacCarty described the earliest cytologic changes in the tubules under the term secondary cytoplasia.[9] By a close study of these changes in the tubules it is possible to differentiate easily between heterotropic glands and early cancer.[10]

DIFFERENTIAL POINTS IN HISTOLOGIC DIAGNOSIS

It is probably a mistake to speak of the degeneration of ulcer into cancer. Cancer is biologically a defensive constructive process, although

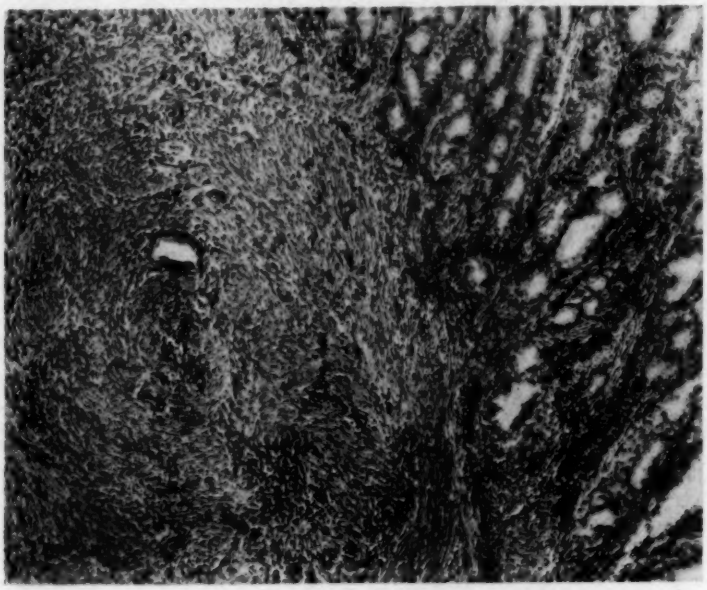

Fig. 4.—Normal heterotropic gland in submucosa.

for the organism as a whole it forms a purposeless, functionless and eventually fatal new growth. The cancer cell is an undifferentiated or partially differentiated cell and the greater the amount of differentiation the lower the malignancy; the less the differentiation the higher the malignancy.[11]

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9. MacCarty, W. C., and Broders, A. C.: Chronic Gastric Ulcer and Its Relation to Gastric Carcinoma, Arch. Int. Med. **13**:208 (Feb.) 1914.

10. Maniscalio, Giuseppe: Sulla etiologia e patogenesi del cancro, Riforma med. **21**:340, 1905.

11. Broders, A. C.: Squamous-Cell Epithelioma of the Lip: A Study of Five Hundred Thirty-Seven Cases, J. A. M. A. **74**:656 (March 6) 1920; Squamous-Cell Epithelioma of the Skin: A Study of 256 Cases, Ann. Surg. **73**:141, 1921; The Grading of Carcinoma, Minnesota Med. **8**:726, 1925.

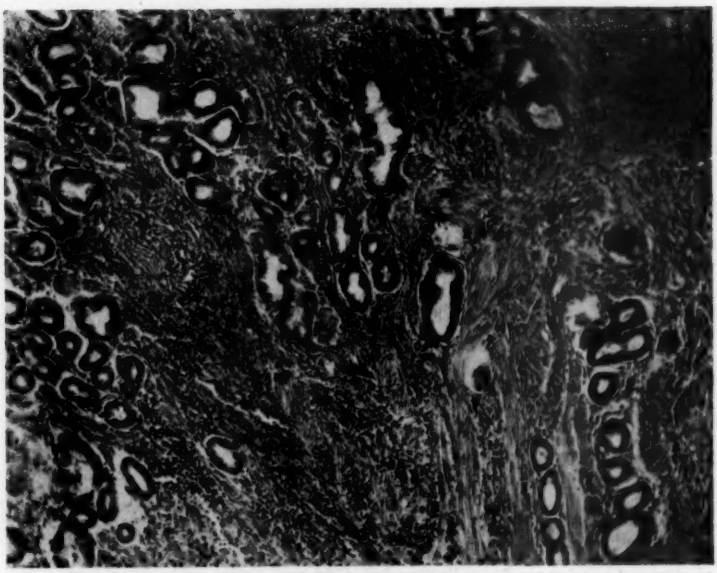

Fig. 5.—Heterotropic glands in muscularis mucosae and submucosa with some atypical epithelial cells; × 75.

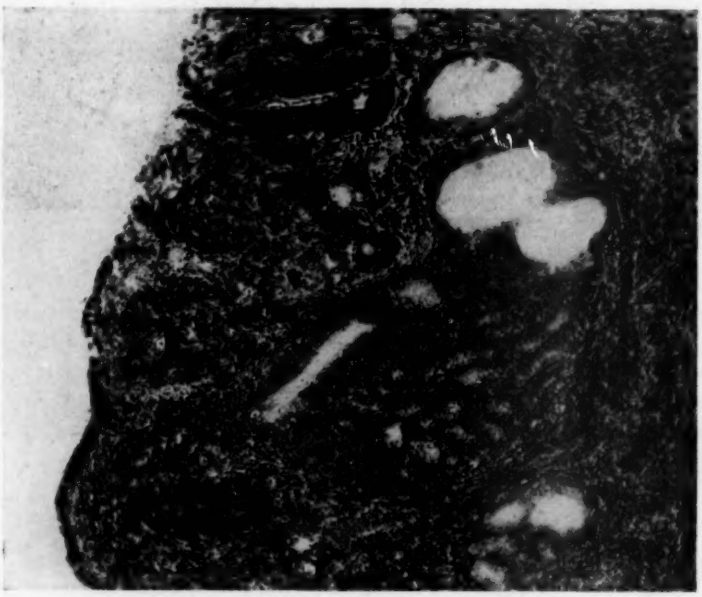

Fig. 6.—Section of ulcer shown in figure 2; early cancer limited to the mucosa is shown.

In carrying out this routine of differential diagnosis of benign and malignant ulcers, the highest powers of the microscope must be used, because malignant and regenerative cells resemble each other closely.[12] Both cells are spheroidal or ovoidal and usually larger than a normal epithelial cell. The nuclear membrane of the regenerative cell is more delicate and the size of the nucleolus in proportion to the volume of the nucleus is smaller, whereas the nuclear membrane of the malignant cell is more dense and the nucleolus, which is often multiple, is hyperchromatic and larger in proportion to the volume of the nucleus.[13] In sinuses of lymph nodes, the distinction must be made from lymphoblasts, endothelioblasts and fibroblasts. In the tubules of the gastric

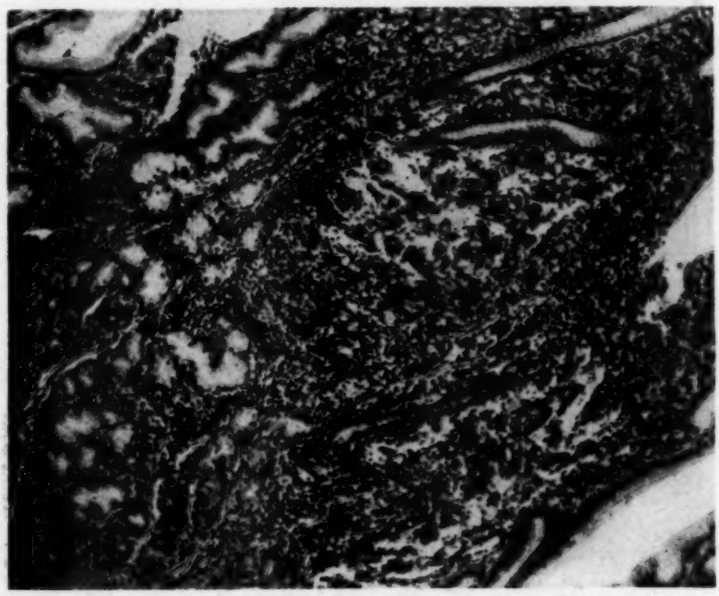

Fig. 7.—Section of ulcer shown in figure 3; early cancer limited to the mucosa is shown.

mucosa, regenerative cells occupy a position which is perpendicular to the plane of the stroma; their long axes radiate from the center of the tubule like the spokes of a wheel. Malignant cells, on the contrary, are irregularly arranged in relation to each other and the plane of the stroma, with the long axes usually parallel to or bent toward the basement membrane. The malignant cell is indistinguishable from the cells of secondary cytoplasia.[13]

12. MacCarty, W. C.: The Histogenesis of Cancer of the Stomach, Am. J. M. Sc. **149**:469, 1915.

13. MacCarty, W. C.: The Cancer Cell and Nature's Defensive Mechanism, Surg. Gynec. Obst. **41**:783, 1925.

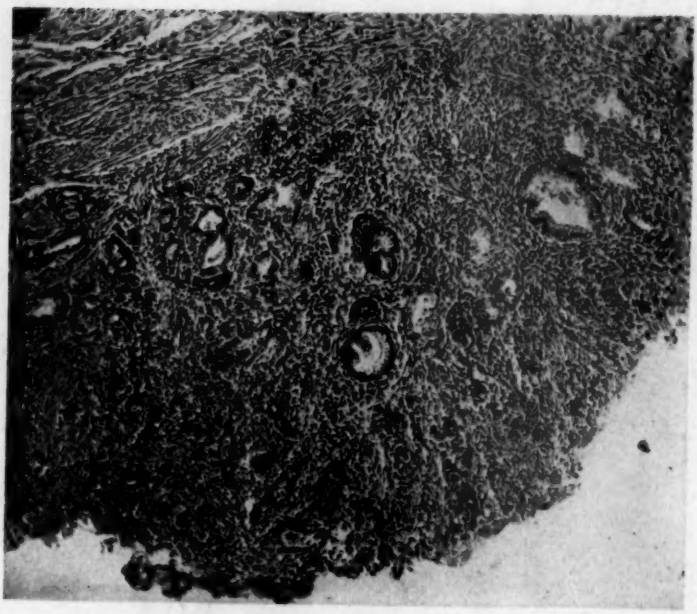

Fig. 8.—Border of ulcer; cancer in mucosa is shown; × 75.

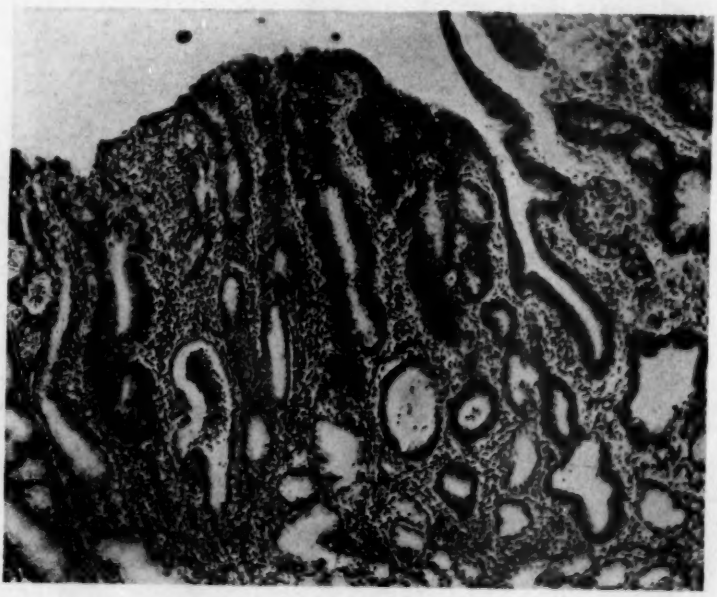

Fig. 9.—Border of ulcer; cancer in mucosa is shown; × 75.

The earliest changes must be sought for in the cells lining the tubules of the gland. If the cells of secondary cytoplasia are seen breaking through into the stroma, the condition is termed tertiary cytoplasia.[14] Ulcers in which secondary cytoplasia was found alone have not, in this study, been considered malignant. But if in the borders of small and large ulcers tertiary cytoplasia, in addition to secondary cytoplasia, was found, this condition has been designated early cancer.

In the illustrations of early cancer are seen the cytologic changes described (figs. 1 to 10); this malignant change is entirely limited to the mucous membrane. Secondary cytologic changes are present with some of the growth or proliferation and migration, showing the formation of new glandular tissues. Various so-called types of cancer are

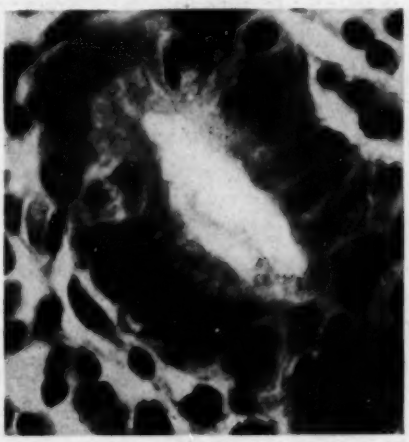

Fig. 10.—Cells having the appearance of malignant cells within the tubule; × 700.

seen: the type composed of simple rather small cells diffusely growing, the glandular or adenomatous type and the adenomatous-colloid type.

CONCLUSION

Differential clinical diagnosis of benign and malignant gastric ulcers is notoriously defective.[15] All chronic callous gastric ulcers are suspected of being carcinomatous and should be treated as such before, and at the time of, operation. The use of the microscope is the only means of distinguishing simple chronic gastric ulcer from early gastric cancer. The diagnosis cannot be made by clinical means, roentgenoscopy or the appearance of the gross specimen.

14. MacCarty, W. C.: A Biological Conception of Neoplasia, Its Terminology and Clinical Significance, Am. J. M. Sc. **157**:657, 1919.

15. Mayo, W. J.: The Calloused Ulcer of the Posterior Wall of the Stomach, Ann. Surg. **72**:109, 1920.

ERYSIPELAS OF THE STOMACH *

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Recently, we had the opportunity of observing before death, and finding at autopsy, a case of primary idiopathic phlegmonous gastritis of streptococcal origin in which the gross appearance of the stomach suggested to one of us (M) the possibility of its being erysipelas. The histologic changes were identical with those seen in erysipelas of the skin or that of mucous membranes, and the streptococcus occurring in enormous numbers in the submucosa was positively identified by Birkhaug as belonging to the group of *Streptococcus erysipelatis.*

Phlegmonous gastritis is said to be a rare disease. Known since the time of Galen, it was first accurately described in 1820 by Cruveilhier.[1]

In 1919, Sundberg [2] published a review of 215 case reports which he had collected from the literature. In 1927, Gerster [3] added forty-eight additional case reports, making a total of 263 recorded up to 1927. Most cases, however, are never reported. In any large autopsy service, the various types of phlegmonous gastritis and enteritis, while uncommon, occur with such frequency as to be hardly considered rare.

The cases reported have usually occurred in persons between the ages of 20 and 60 years. The disease is about three times as frequent in men as in women.

Phlegmonous gastritis occurs in diffuse form, extending over the entire stomach wall, or in localized, circumscribed form. Of the 185 reports of cases of phlegmon in Sundberg's collection, 158 were of the diffuse, and twenty-seven of the circumscribed, form. Streptococcus is by far the commonest organism recovered (in 70 per cent of the cases). Staphylococci, pneumococci, *Bacillus coli* and *B. subtilis* have been isolated in a few cases, usually of the localized form. Some of the reported cases were associated with old or recent lesions in the mucosa

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* Submitted for publication, Sept. 5, 1929.

* From the pathological department of the City Hospital, and the office of the Chief Medical Examiner of Essex County, N. J.

* Read before the New York Pathological Society at the New York Academy of Medicine, New York, Nov. 8, 1928.

1. Cruveilhier: Traité d'anatomie pathologique en générale, Paris, 1862, vol. 4, p. 485.

2. Sundberg, H.: Nord. med. ark. **51**:303, 1919.

3. Gerster, J. C. A.: Phlegmonous Gastritis, Ann. Surg. **85**:668 (May) 1927.

of the stomach, particularly with ulcer and carcinoma. Others were said to have followed abdominal blows or trauma. Cases of the primary idiopathic form, which is the most common and most important, showed no gross lesion in the mucosa and were considered by MacCallum [4] and others as definitely streptococcal in origin. The submucosa of the stomach was found to be enormously thickened by a tense inflammatory exudate loaded with streptococci.

According to Gerster,[3] the typical symptoms of the so-called idiopathic form are "sudden onset, with profound prostration, high fever, chills, intense epigastric pain and tenderness, repeated severe vomiting and more or less local rigidity." Peritonitis occurs in from 60 to 70 per cent of the cases. Diagnosis is rarely made before operation, the condition usually being mistaken for acute perforated gastric ulcer, acute pancreatitis or acute cholecystitis.

Phlegmonous gastritis in most cases is an acute lesion and usually fatal. Recovery, however, may take place. The average duration of the disease is one or two weeks, but in several cases death has occurred within a few hours of onset. The mortality is 92 per cent (Sundberg). The disease also occurs in subacute and chronic forms. Such cases are often localized, not extensive and usually associated with ulcer or carcinoma. Numerous eosinophils may be present in the exudate. Whether a benign form of leather bottle stomach (linitis plastica) may result from the healing of localized or diffuse forms is questionable, as most cases of this disease are really sclerosing fibrocarcinomas of the stomach. It is possible, however, that some cases of hour-glass stomach represent the end-stages of healed phlegmons.

The absence, in most cases, of any obvious portal of entry to explain the infection has forced most recent observers to fall back on the hematogenous routes. Some authors also give as explanation of the infection the more fashionable, but equally vague and unproved, selective affinity of streptococci for certain tissues. Phlegmonous gastritis has occasionally been observed during epidemics of puerperal sepsis, notably one in Prague in 1847. Cases have also been seen following erysipelas or furunculosis, or as sequelae to smallpox, scarlet fever, polyarthritis and pyemia. They sometimes occur as a postoperative complication.

To our knowledge, phlegmonous gastritis has never been interpreted as erysipelas of the stomach, with perhaps one exception. Sachs [5] stated that in the pathologic museum of the Mount Sinai Hospital, New York, there is a specimen of phlegmonous gastritis to which it was Libman's

4. MacCallum, W. G.: Textbook of Pathology, ed. 4, Philadelphia, W. B. Saunders Company, 1928, p. 489.

5. Sachs, Benjamin: In discussion of authors' paper at meeting of New York Pathological Society, Nov. 8, 1928.

practice to refer as erysipelas of the stomach. Libman [6] informed us that this occurred in a patient who five weeks before death had an erysipelas of the leg. He was of the opinion that the lesion looked like erysipelas. At that time (1909), little was known concerning the serologic grouping of the streptococci.

Aside from this case, we are unable in a careful search of the literature to find any evidence that erysipelas of the stomach has ever been described. Erysipelas commonly occurs on the face, head, scrotum and legs; around surgical wounds and around the navel of new-born infants. It is a well known fact, however, that erysipelas is not confined to the skin. Holmes [7] held that most cases of facial erysipelas start in the mucosa of the nasal cavities, possibly from latent infection of the nose, nasal sinuses, etc. Erysipelas may often extend to the mucous membranes of the mouth, nose, eyes, pharynx, middle ears, larynx, rectum and vagina. From the mouth it has sometimes spread to the lungs, pharynx and esophagus. The frequent infection of women during childbirth has long been recognized, as well as the danger of the erysipelatous process spreading to the uterine mucosa and adnexa.

While phlegmonous gastritis is unusual, we do not feel that this warrants the report of a case unless there is some outstanding new feature. A case in which the gross appearance and histologic features are identical with erysipelas and in which, for the first time, there is bacteriologic and serologic identification of the organism present, as of the group of *Streptococcus erysipelatis,* warrants, we believe, such a record.

REPORT OF CASE

History.—E. H., a white woman, aged 72, was admitted to the Newark City Hospital in a dying condition. Seven days before death, the patient was suddenly seized with cramplike pains localizing in the upper left quadrant of the abdomen. She was nauseated and she vomited twice. Pain became intense. Two days later, the family physician was called in, and the pain was relieved somewhat by opiates. Five days later, the patient was again seen by the family physician, who found the abdomen markedly distended and tympanitic. The bowels had not moved for five days. There was tenderness in the left hypochondrium extending into the epigastrium, and on palpation the sensation of a tumor mass. On account of no improvement in the general condition of the patient, she was sent to the hospital, where she lived only five hours. Examination at the hospital showed an elderly white woman in a moribund condition. There was great distention and tenderness all over the abdomen. Only stimulative treatment was administered. A clinical diagnosis of generalized peritonitis, possibly due to a malignant abdominal growth, was made.

Autopsy.—In order to obtain permission for an autopsy, the undertaker was allowed to embalm the body by the arterial method. This, however, did not seri-

6. Libman, Emanuel: Personal communication to the authors.

7. Holmes, Charles: Ann. Otol. Rhin. & Laryng. **16**:457, 1907.

ously interfere with the correct interpretation of the lesions, as the autopsy was performed immediately afterward. Autopsy showed the body of a somewhat emaciated woman, aged 72, with a greatly distended abdomen. There were no marks of injury on the skin, no rash and no edema. Aside from cloudy swelling in the heart muscle, liver and kidneys, a terminal pulmonary edema and vascular changes due to age, the important observations were those of the stomach and peritoneum.

The stomach was greatly distended. Near the pylorus, it felt as if there was a large stenotic growth. The whole stomach wall was leathery and did not collapse. There were no enlarged glands among the lesser or the greater curvature, and no enlargement of the periportal nodes. The esophagus was free. On opening the stomach, which was empty, one saw a remarkable condition. There were marked thickening and edema of the stomach wall, most marked in the pyloric

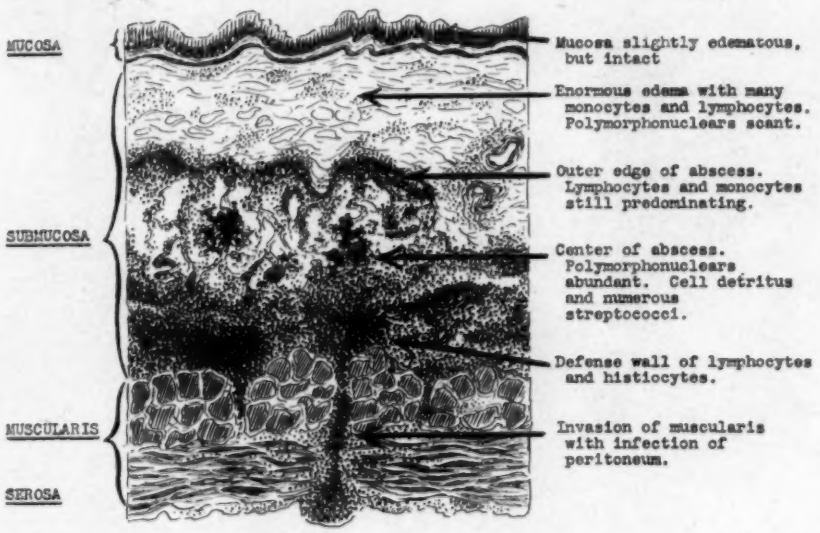

Fig. 1.—Schematic diagram of stomach wall showing the location and character of the lesion.

half, where the wall was ½ inch (1.27 cm.) thick. The mucosa, in spite of the fact that it was bleached by the embalming, was still a diffuse scarlet hue, and showed only an occasional mucosal and submucosal hemorrhage. The rugae were obliterated and ironed out by the edema. There were no recent or old ulcers and no macroscopic abrasions. Close inspection of the stomach wall showed that over one half of the thickening was due to distention of the submucosa by a watery, purulent exudate. The mucosa was but slightly swollen. In the submucosa, lying between the mucosa and the internal circular muscular coat, which was plainly visible, there was a large amount of grayish, watery, purulent fluid which could be pressed out. The submucosa had a somewhat honey-combed appearance due to a partitioning off of this purulent fluid by connective tissue trabeculae. In places, multiple small abscesses were seen in which the fluid was thicker and more creamy. Grossly, the muscularis, aside from the edema, was free from changes. Over the serosa was a plastic purofibrinous exudate. This

suppuration extended diffusely thorughout the entire submucosa of the stomach, but was more pronounced in the pyloric half. It was sharply demarcated above by the esophageal orifice and below by the pyloric valve. The mucous membrane of the esophagus and duodenum appeared normal. There was considerable purulent fluid in the pelvis and abdominal gutters and there was extensive fibropurulent exudate over the stomach and loops of small intestine, gently gluing them together in places.

Death was caused by phlegmonous gastritis, with terminal generalized suppurative peritonitis through extension from the stomach by continuity.

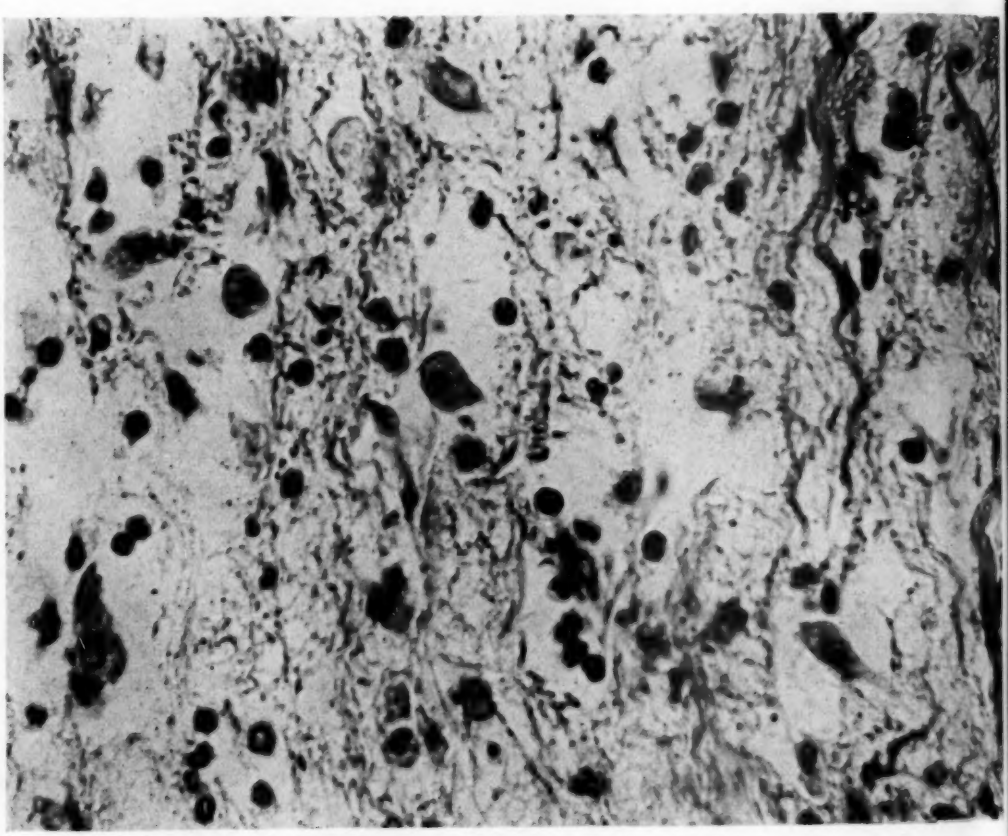

Fig. 2.—Photomicrograph showing inner portion of the submucosa. The distention of the tissue spaces with edema and the presence of an exudate composed chiefly of lymphocytes and monocytes may be noted. Streptococci were not numerous in these areas; × 480.

Bacteriology.—Smears made from the fluid pressed from the submucosa of the stomach showed a great number of gram-positive streptococci. No other organisms were present.

As the body had just been embalmed, the isolation of this streptococcus was expected to be difficult. However, by transferring portions of the stomach wall over in large quantities of broth we finally obtained, in pure culture, a hemolytic, long-chained streptococcus.

Histology.—Microscopic sections were made through the entire thickness of the stomach wall. The mucosa was shown intact and was only slightly edematous; it was practically free from any cellular exudate. The muscularis mucosa was intact, but in places it was edematous and swollen, and showed a slight infiltration with lymphocytes and histiocytes. The submucosa was greatly thickened, forming over one half of the entire thickness of the stomach wall. Its inner portion was comparatively free from cellular exudate, but there was enormous edema, the fluid distending the tissue spaces and widely separating them. The little cellular

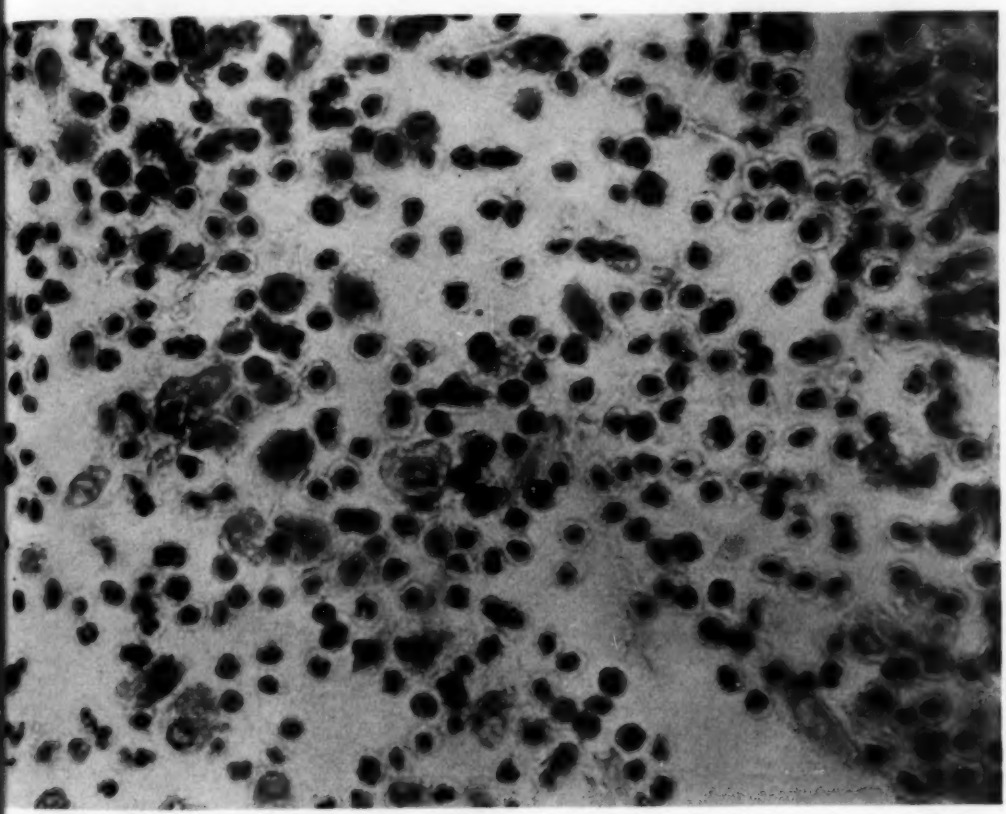

Fig. 3.—Photomicrograph showing deeper portions of submucosa. The extensive suppuration may be noted. While numerous polymorphonuclears are present, the lymphocytes and monocytes predominate. Numerous streptococci are present in these areas; × 480.

exudate present was composed chiefly of lymphocytes and monocytes. No definite relationship to the lymphatics could be made out. Few polymorphonuclears were present. As one approached the middle of the submucosa, the exudate became abundant, and more polymorphonuclears were present. This became so marked as to form small abscesses. The periphery of these areas was formed chiefly of lymphocytes and monocytes, and the central portions contained chiefly polymorphonuclears. In the center of many of these areas there was extensive necrosis

with numerous polymorphonuclears and considerable cellular detritus. Gram stains showed numerous streptococci in these areas, and they were scant or absent in other portions of the stomach wall. Near the internal circular layer of the muscularis there was a strong defense wall composed of many histiocytes and lymphocytes and few polymorphonuclears. In places, this cellular exudate filtered through the muscle bundles of the muscularis and extended to the serosa, where there was edema with beginning suppurative peritonitis. The muscularis itself was free. Practically no eosinophils were present.

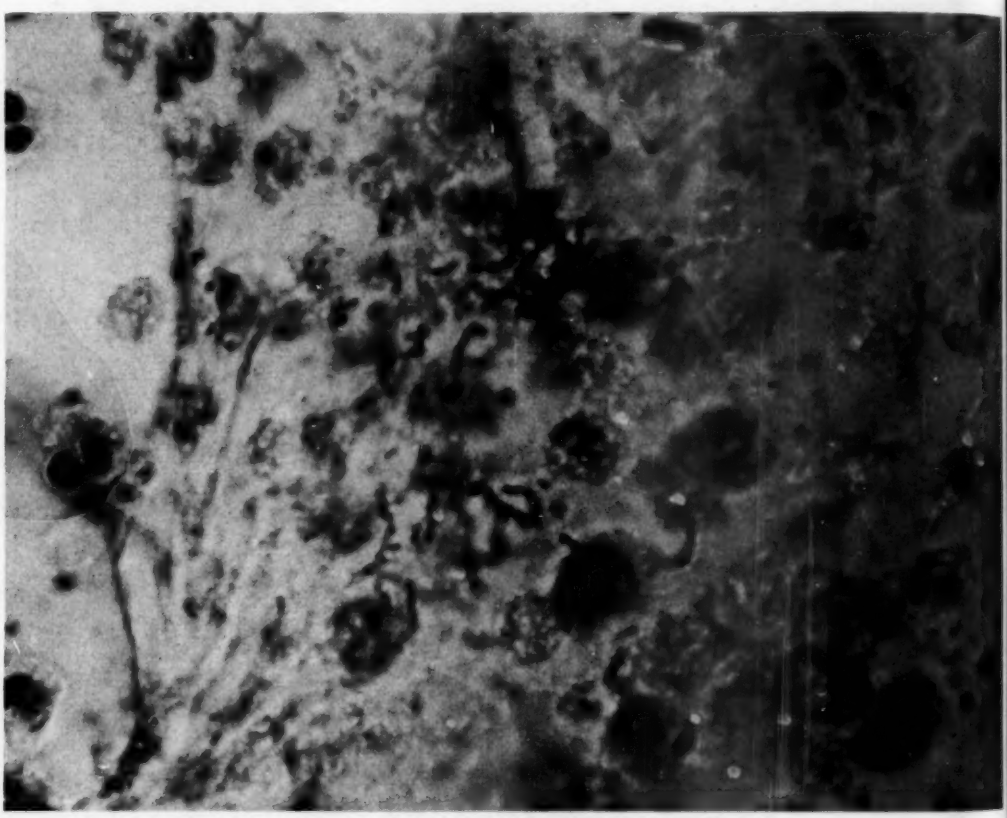

Fig. 4.—Photomicrograph taken from a central area of suppuration in the submucosa. Necrosis, cell detritus and numerous streptococci may be noted. Gram-Weigert stain; × 1,050.

IDENTIFICATION OF STREPTOCOCCUS BY BIRKHAUG

An agar slant containing a pure culture of a gram-positive hemolytic streptococcus recovered from the submucosa of the stomach at autopsy by Dr. Lloyd Riggs, assistant bacteriologist to the Newark Department of Health, was sent to Dr. Konrad E. Birkhaug, associate professor of bacteriology, University of Rochester, for identification.

Birkhaug was not able to agglutinate this culture properly with his standard erysipelas immune serums because of its quick spontaneous precipitation in broth during the first ten hours of growth. He resorted then to the absorption of known antibodies for *Streptococcus erysipelatis* and found that the strain removed the agglutinins for three of four of his standard strains of *S. erysipelatis.* He repeated this process twice and obtained identical results. Birkhaug also attempted to remove the agglutinins in the antiserum for *Streptococcus scarlatinae* with this strain and found that the original titer of 1:2,560 was reduced only to 1:1,280 with the homologous strain of *S. scarlatinae* (Dochez, N. Y. 5). The final criterion of typical *S. erysipelatis* is its constant production of toxin in Douglas' tryptic digest broth. The five days' culture of this strain was filtered through a Berkefeld V candle. In a series of titrations in the skins of persons susceptible to the toxin of *S. erysipelatis,* he found that a potent exotoxin was produced, 1 cc. of which contained more than 10,000 skin test doses. In proper dilutions with the antitoxin for *S. erysipelatis,* this toxin was completely neutralized. Putting these facts together, Birkhaug stated, "It appears that your strain is definitely related to the serological types of Streptococcus erysipelatis."

This is the first time, therefore, that the streptococcus isolated from a case of phlegmonous gastritis has been positively identified as belonging to the erysipelatis group. This is due entirely to the painstaking, classic identification by Birkhaug.

CONCLUSIONS

A case of primary idiopathic phlegmonous gastritis has been described, in which the appearance of the stomach was identical with that of the stomach in most cases described in the literature. There were no gross lesions of the mucous membrane, such as ulcer, carcinoma, abrasions or other injuries. The inflammation was diffuse, extending over the whole stomach, but limited almost entirely to the submucosa. The diffuse blushing of the mucosa, the ironing out of the rugae by edema and the thick, edematous condition of the submucosa filled with a watery, grayish fluid containing innumerable streptococci with occasional small abscess formations suggest the gross appearance of erysipelas, and the picture is similar in every way to that of extensive erysipelas of the skin with cellulitis of the subcutaneous tissues.

The sharp demarcation of the suppurative process in this case by both the cardiac orifice and the pylorus, both of which have a mucosa which is rather tightly bound down, is similar, for instance, to the sharp demarcation and stoppage of facial erysipelas at the lower border of the mandible.

Histologic examination showed the lesion to be almost entirely confined to the submucosa. It is characterized by extensive boggy edema, in which the cellular exudate is composed chiefly of lymphocytes and monocytes. The polymorphonuclears are abundant only in the central areas, where formation of abscesses occurs, and where there is necrosis, with cell detritus and innumerable streptococci. This histologic picture is identical with that seen in erysipelas.

The identification of the streptococcus by Birkhaug, as belonging serologically to the group of *Streptococcus erysipelatis,* completes the case and proves, as nearly as can be, that some forms of phlegmonous gastritis may be considered erysipelas.

CUTANEOUS LESIONS (INFECTIOUS GRANULOMAS) ON THE FEET OF ALBINO RATS*

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AND
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The occurrence of well defined ulcers on the bony prominences of the lower forelegs of dogs maintained on diets deficient in vitamin B complex has been reported by Cowgill, Stucky and Rose (1929 [1]); these lesions healed promptly when this food factor was replaced. The parallelism between the occurrence of the lesions and the dietary deficiency was definitely suggestive. The authors noted that the ulcers did not appear in all the animals subjected to the experiment. Furthermore, they noted scars of presumably identical lesions in other dogs that were not subjects of experiments in nutrition. They recognized that the factors of pressure and local injury were necessarily uncontrolled variables. The purpose of this communication is to report the occurrence of lesions on points of pressure on the feet of white rats maintained on adequate diets.

The photographs accurately portray the lesions. They usually occurred in the situation shown in the illustrations. Only in a few instances were they observed on the forefeet and on the anterior portion of the hind feet. As a rule, they were bilaterally symmetrical.

In the early stages, they appeared as slightly elevated, pale red, subepidermal nodules. The overlying epidermis was intact. The following changes then occurred successively: loss of the overlying epithelium, formation of a superficial ulcer with a base of granulation tissue, and finally a marked overproduction of granulation tissue. The surfaces of the lesions were deep red and relatively free from exudate, and bled after slight trauma. In a few instances, the productive nature of the lesions was less evident, the bases of the ulcers being flat or somewhat depressed (fig. 1).

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* Submitted for publication, Sept. 5, 1929.

* From the Laboratory of Physiological Chemistry and the Department of Surgery, Yale University.

* Aided by a grant from the Committee on Scientific Research of the American Medical Association.

1. Cowgill, G. R.; Stucky, C. J., and Rose, W. B.: Physiology of Vitamins: Cutaneous Manifestations Related to Deficiency of Vitamin B Complex, Arch. Path. **7**:197 (Feb.) 1929.

The lesions were generally oval or spherical and sometimes were pedunculated or sessile. The shape was probably determined by such external factors as the location, the pressure and the amount of irritation.

Microscopic sections confirmed the gross observations. The earliest change was a slight thickening of the dermis, which was followed by a loss of the overlying epidermis, the formation of an ulcer with a base of granulation tissue and finally the overgrowth of granulation tissue to

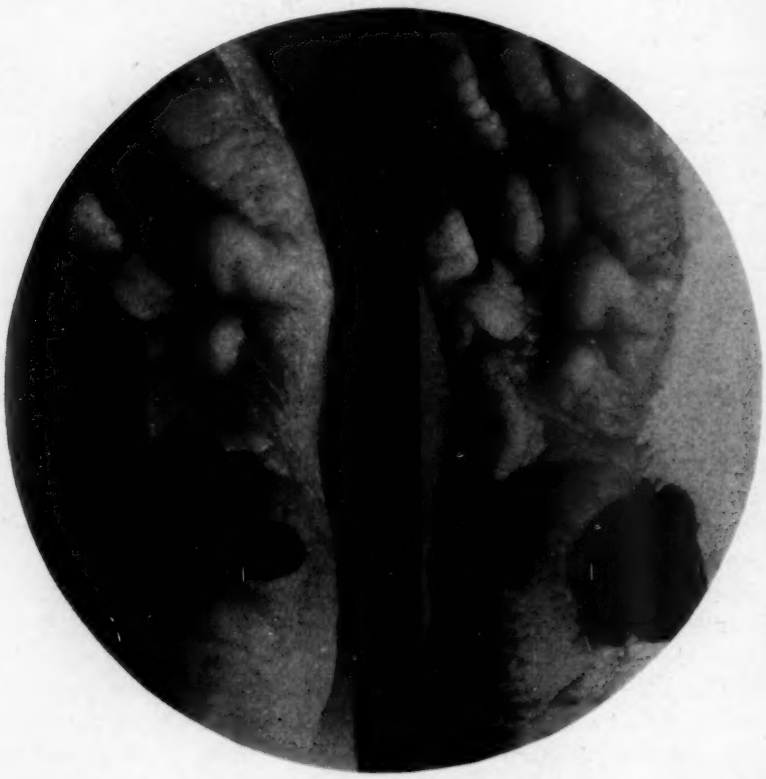

Fig. 1.—An ulcerative lesion (left) and a proliferative lesion in profile (right); × 2½.

form a protuberant lesion. In some instances, a typical depressed ulcer with a flat base developed. There was a thin layer of a fibrinopurulent exudate on the external surfaces and an infiltration of the granulation tissue by polymorphonuclear leukocytes.

The character of the granulation tissue varied with the age and the size of the lesions. In the more advanced stages, the superficial portions showed a delicate meshwork of capillaries, but the deeper areas showed a large amount of fibrous tissue. The essential nature of the proliferative lesions was that of an infectious granuloma (figs. 2 and 3).

The larger granulomas showed some evidence of epithelial proliferation, although there was nothing suggestive of epithelial invasion of the surrounding tissue.

The lesions in our animals were similar to the granuloma pyogenicum of man (Michelson, 1925 [2]), the most characteristic feature of which is the proliferation of the granulation tissue to form a small pedunculated or sessile nodule. They differ from the lesions described by Cowgill, Stucky and Rose in that the latter were definitely depressed

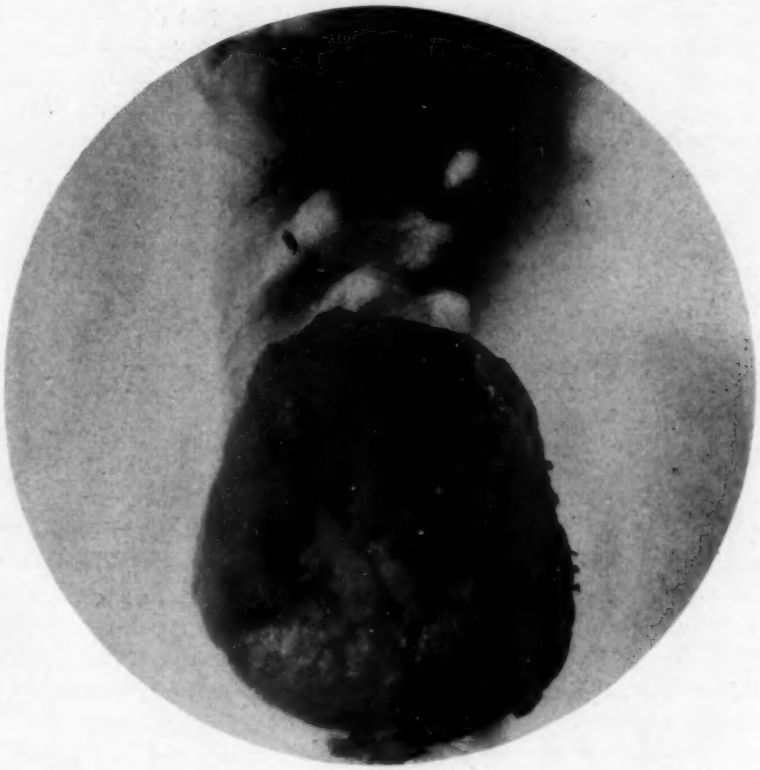

Fig. 2.—Large granuloma in the advanced stage; × 2½.

ulcers without evidence of granuloma formation. In some instances, similar ulcers were observed in our animals. The differences in the nature of the ulcerative and granulomatous changes may be due to such variable factors as species of animal, duration of experiment and infection.

The observations here recorded were made on a large series of rats used in an experiment in nutrition. Three adequate rations were

2. Michelson, H. E.: Granuloma Pyogenicum: Clinical and Histologic Review of 29 Cases, Arch. Derm. & Syph. **12**:492 (Oct.) 1925.

employed varying only in the concentration of protein (casein) as follows: 18, 60 and 85 per cent. Most of the animals had been subjected to unilateral nephrectomy; a few were controls on which laparotomy only had been performed. There were three age groups at the time of operation—90, 180 and 360 days—and the experimental period extended for 56 days and 150 days longer. The rats were kept in cages with wire screen bottoms during their entire life.

At the outset, it can be said that the lesions occurred irrespective of the diet consumed. The rats that were 90 days old when the experiment began were practically free from lesions; those 180 days old showed early changes rather uniformly, and the animals 360 days of age exhibited severe ulcers in almost every case. These were more advanced in the 150-day, than in the 56-day, period. It appears, therefore, that the incidence of the lesions is correlated with the factors accompanying age rather than with diet. As the older rats are heavier, it would seem that

Fig. 3.—Longitudinal section through the granuloma shown in figure 2. The lighter portions near the base are areas of epithelial proliferation; × 2½.

pressure on the feet and the length of time are important factors in the pathogenesis of these ulcers. It is possible, too, that the tissues of older animals are rendered more susceptible to injury during senescence.

In view of the dietetic adequacy of the rations employed and the absence of any evidences of nutritive disturbance in the animals here discussed, it is suggested that caution be exercised in deciding whether or not the ulcers observed on bony prominences of animals maintained for relatively long periods under experimental conditions necessarily have a specific dietary significance. On the other hand, it is reasonable to expect that a nutritive disturbance might lower the resistance of body tissues to such an injury as that described and that improvement in the local lesions would be observed along with disappearance of the general manifestations of the deficiency.

The lesions described in the present communication are probably not due to the lack of a specific nutritional factor, but to repeated slight traumas and a superimposed infection with pyogenic organisms.

MULTIPLE SPLEEN COMBINED WITH OTHER CONGENITAL ANOMALIES

REPORT OF TWO CASES *

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Accessory spleens are not infrequently encountered at necropsy. I have seen them in about 10 per cent of bodies during routine postmortem examination. They are small and usually single, and the spleen itself is normally developed. The appearance of multiple spleen without a fully developed spleen is apparently rare.

Albrecht [1] reported 400 spleens in a man, aged 25, with the real spleen situated higher than usual and adherent to the diaphragm. These accessory nodules were scattered all over the peritoneum. Kaufmann [1] mentioned the case of Faltin and Kuttner, in which a splenectomy was done for a gunshot wound that had done crushing injury to the spleen, and five years later about 1,000 spleens were found on the peritoneum. Beneke [2] thought such cases were the result of trauma with splenic regeneration from implantation. Pool [3] mentioned the occurrence of multiple spleens with malformation of other viscera. He cited Hyrtl's four cases, with transposition of the viscera. In these cases, spleens were broken up into from five to eleven smaller nodules. Garrod, according to Pool, reported two cases of heart disease with multiple spleens, 4 and 9, respectively. He likewise mentioned Helly's specimen, which is preserved in the anatomic museum at Vienna; in this there was a bilobular spleen with eleven accessory spleens. Other unusual anatomic variations of great interest have been reported.

The splenic anomalies that I present are rare. I can discover no other example of the condition in the first case, in which there was a combination of multiple spleens with congenital stenosis of the bile ducts and cirrhosis of the liver. The second case parallels somewhat those of Garrod, since it showed a congenital cardiac lesion with bilobular spleen and ten accessory portions.

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* Submitted for publication, June 14, 1929.

* From the Department of Pathology, St. Luke's and Children's Mercy Hospitals.

1. Albrecht, cited by Kaufmann, Edward: Pathology for Students and Practitioners. Authorized translation by Stanley P. Reimann, Philadelphia, P. Blakiston's Son & Company, 1929, vol. 1.

2. Beneke, cited by Kaufmann (footnote 1).

3. Pool, E. H.: Surgery of the Spleen, New York, D. Appleton & Company, 1923.

REPORT OF CASES

Case 1.—C., an infant, was jaundiced at birth. The delivery was easy and spontaneous. The cord came off on the sixth day after birth. On the ninth day, the child began to bleed from the umbilicus. On the twelfth day, she was brought to the hospital with a slow oozing from the navel. The skin and sclera were markedly jaundiced and she was emaciated. The mother admitted having had syphilis, which she had contracted from her first husband. She had been treated and pronounced cured. There was one living child, aged 5. The family history was of no significance otherwise.

The infant received 50 cc. of the father's blood intramuscularly, and four days later was given 150 cc. intraperitoneally. She progressed poorly until about the eighteenth day of life when, following venipuncture of the left external jugular, she began to have rapid respiration and became paler, and the heart beat became rapid. The impression was that the child was bleeding from some hidden site. She also bled considerably from the needle puncture in the neck and there was oozing into the surrounding cervical tissues. The results of the laboratory examinations were negative, except for the fact that the child was shown to have 17,550 white cells per cubic millimeter of blood. No differential white cell counts or red cell counts were made. The Wassermann reaction was negative.

At the necropsy, which was performed shortly after death, the most outstanding external feature was a striking jaundice of the skin, sclera and mucous membranes. There were large areas of hemorrhage in the region of the jugular vein on the neck, where blood had been withdrawn for examination, with slow oozing into the surrounding tissue. There was also a hemorrhage around the umbilicus, and hemorrhages in the tissues of both hips where injections had been made. Apparently there had been little tendency to clot. The hemorrhage was found to extend into the subcutaneous tissues of the neck, from the chin to the clavicle, throughout the left anterior angle of the neck. The blood from the heart had the viscosity of water, and was not clotted. Small hemorrhages were found scattered over the pleura, and in the parenchyma of the lung many were seen. The lungs were not fully expanded. The thymus was atrophied. The main changes otherwise were found in the abdomen. Masses of fetal adhesions were found over the gallbladder, connecting it with the transverse colon and duodenum. The liver was large, measuring 10.5 by 8 by 6 cm., and weighing 156 Gm. It was hard and showed a slightly wrinkled capsule and was deeply jaundiced. On cross-section, it cut with great resistance, and showed a high grade cirrhosis of periportal type. The gallbladder was little more than a fibrous cord with a small lumen. Both the common and the hepatic duct were completely occluded.

Histologically, the liver showed marked fibrosis, with a tendency to a periportal arrangement. Considerable inspissation of bile was noted in the canaliculi and smaller ducts.

The spleen was divided into fourteen separate and distinct nodules by fetal adhesions, these nodules varying in size from 20 to 2 mm. in diameter. Each nodule received a branch of the splenic artery.

Histologically, these spleens showed nothing unusual or particularly abnormal. There were some foci of myeloid cells in the pulp.

Case 2.[4]—W., an infant, was born normally. The mother had had two former pregnancies. The first pregnancy resulted in a stillborn infant. At the second, she bore twins, but they died before birth, the mother having developed a severe

4. Permission to report case 2 was obtained from Dr. Waller Hook.

toxemia and labor having been brought on prematurely by bag induction. The child in question had attacks of cyanosis and shallow breathing, and died about forty-eight hours after birth.

At necropsy, the heart was large, weighing 37 Gm. The right auricle and ventricle were markedly distended and hypertrophied. The left ventricle was small, and no aorta was found leaving it. The foramen ovali was wide, and no valve was present. The pulmonary artery gave branches to the lungs and then

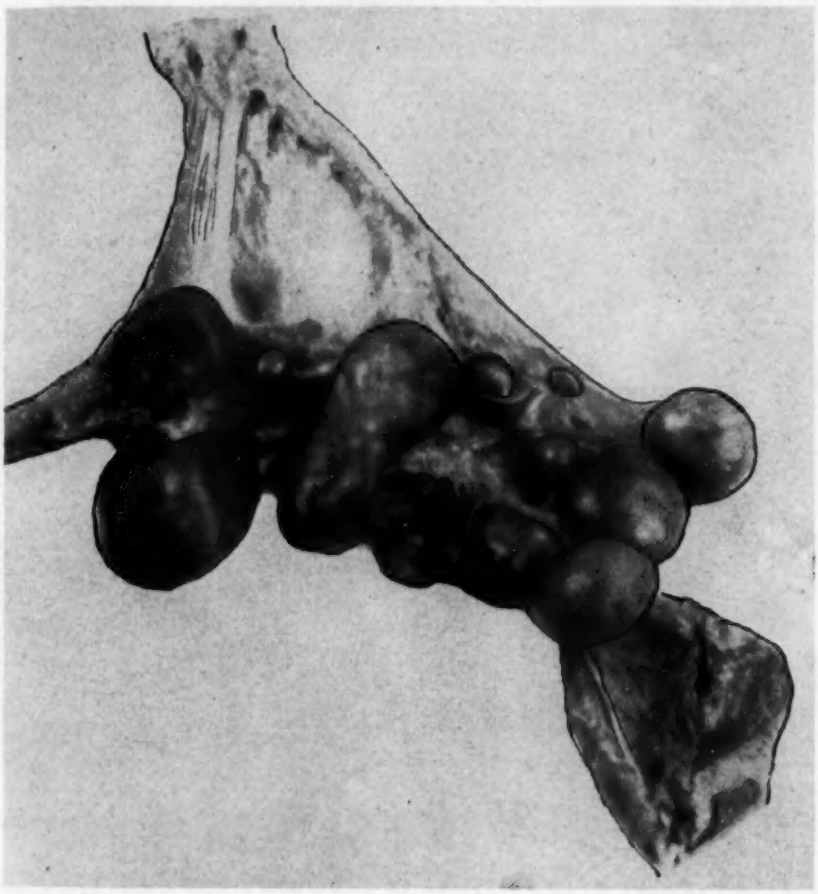

Fig. 1.—Fourteen separate splenic nodules, each with a distinct blood supply from a branch of the splenic artery. Twice natural size.

continued on down, becoming the systemic aorta. The lungs were but partially expanded. All the abdominal viscera showed deep hyperemia, the liver being particularly large. The gallbladder was covered with dense fetal adhesions, which communicated with the transverse colon and the duodenum, as in the first case.

The spleen showed the most interesting picture. It was broken up into ten separate nodules, separated by fetal adhesions, the spleen proper being bilobular and showing three notches. It measured 40 by 22 by 7 mm. The smaller nodules varied from 15 to 2 mm. in diameter.

COMMENT

The etiology of these anomalies is unknown. Since the spleen is not, embryologically, a lobulated organ, such malformations are difficult to explain. Mention was made by Kaufmann [1] of experiments on monkeys in which multiple spleens were produced through traumatization of the original spleen, the regeneration taking the form of multiple nodules. Faltin and Kuttner's case of multiple spleens following a crushing injury to the spleen, and the subsequent growth of 400 implants, is

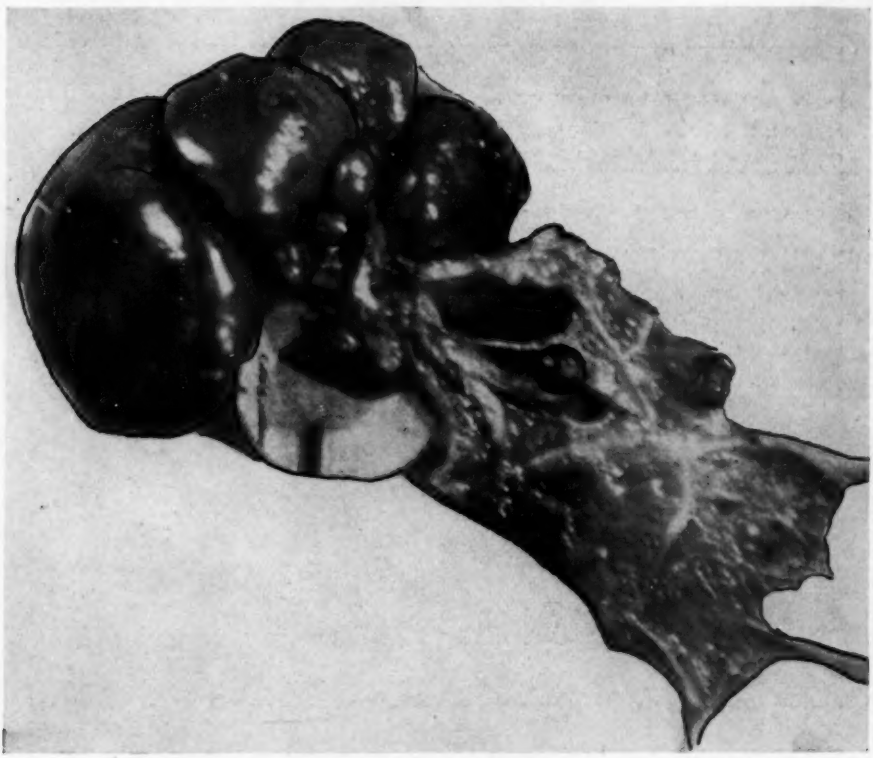

Fig. 2.—Ten separate splenic nodules, each supplied with a branch of the splenic artery. Twice natural size.

suggestive. The presence of fetal adhesions around the gallbladder and the spleen in both my cases suggests a possible intra-abdominal fetal injury.

SUMMARY

Two cases of multiple spleen are reported, one case showing fourteen separate nodules, the other ten. One case occurred in conjunction with congenital stenosis of the extrahepatic bile ducts and the other in connection with a congenital cardiac malformation.

Both cases showed fetal adhesions dividing the lobules and many dense adhesive bands in the gallbladder region. This suggests a possible intra-abdominal fetal injury as an etiologic factor in the production of these anomalies.

RENAL INSUFFICIENCY IN AMYLOID DISEASE*

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AND
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ST. PAUL

Renal insufficiency in amyloid disease has been observed for many years, and contracted kidneys in amyloid disease were first described by Wagner in 1861.[1] Since that time many cases of contracted kidney have been recorded in which the outstanding feature was a massive deposit of amyloid in the glomeruli, in the vessel walls and inside the basement membranes of the collecting tubules. Many of these cases, however, were recorded before the study of blood chemistry was a recognized clinical procedure, while others give no indication that blood chemistry was studied or other tests of renal function performed, the evidence of renal insufficiency being based entirely on anatomic and strictly clinical evidence of kidney damage. The earliest of these reports were from Klebs,[2] and other cases were reported by Schalong,[3] Fahr,[4] Wegelin [5] and Danisch.[6]

There seem to be three generally accepted conceptions of this pathologic entity. The first was advanced by Fahr,[7] who believed that the amyloid kidney is a form of nephrosis. He divided the morphologic changes in the amyloid kidney into four stages, with case reports illustrating each stage. In the cases classified as representing the first stage, he found only a minimal amount of amyloid in the glomeruli, together with a moderate degree of albuminous degeneration of the tubules. As representing stage two, he described cases that showed a larger amount of amyloid in the glomeruli, and more marked degenerative changes in the tubules. These degenerative changes included hyaline granules in the renal epithelium and a rather marked deposit of lipoid, comparable to that seen in lipoid nephrosis. In the

* Submitted for publication, June 1, 1929.

* From the Department of Pathology, University of Minnesota, Minneapolis, and Ancker Hospital Laboratory, St. Paul.

1. Wagner, E.: Arch. d. Heilk. **2**:481, 1861.

2. Klebs, E.: Handbuch der pathologischen Anatomie, Jena, Gustav Fischer. 1889, vol. 2, p. 623.

3. Schalong, H.: Virchows Arch. f. path. Anat. **257**:15, 1925.

4. Fahr, Theodore: Virchows Arch. f. path. Anat. **248**:323, 1924; Berl. klin. Wchnschr. **55**:993, 1918.

5. Wegelin, C.: Schweiz. med. Wchnschr. **56**:716, 1926.

6. Danisch, H.: Verhandl. d. deutsch. path. Gesellsch. **20**:307, 1925.

7. Fahr, Theodore: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1925.

group that he classified as representing stage three, the kidneys contained large amounts of amyloid in the glomeruli with a consequent narrowing of the capillaries. He stated that in these cases, in spite of the narrowing of the capillaries, many of the glomeruli were still permeable to blood. In the fourth stage, the glomeruli were destroyed, and there was a marked tubular atrophy, which was secondary to the glomerular damage.

Fahr believed that the disease is a toxic process affecting both tubules and glomeruli simultaneously, the primary damage being done to the glomeruli. He also described arteriosclerosis of the vessels as occurring particularly in those cases in which the patients were over 50 years of age, but he thought that this change does not play an important part in the production of contracted kidneys in amyloid disease. Fahr's ideas were closely followed by Danisch [6] in his report of two cases of contracted kidney in amyloid disease. Linder,[8] who reported the best studied case we have been able to find in the literature, agreed with Fahr as to the nephrotic character of the disease. Ophüls [9] and McElroy [10] subscribed to the same theory.

The second conception is that amyloid degeneration occurs in the course of chronic nephritis as expressed by Osler [11] and McCrae,[12] it is "simply an event in the process of a chronic nephritis, most commonly in the chronic parenchymatous nephritis, following fevers or cachectic states." McCrae stated that "amyloid kidney is usually spoken of as a variety of nephritis, but in reality it is a degeneration which may accompany any form of nephritis." MacCallum [13] also believed that amyloid is deposited in "any or all of the changes described in progressive nephritis."

MacCallum also stated, however, that amyloid may appear in an otherwise normal kidney in the course of a general amyloidosis. This idea represents the third conception of the disease, namely, that it is simply a part of a systemic condition in which the damage to the

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8. Linder, G. C.; Maxwell, J., and Green, F. H. K.: Clinical, Pathological and Biological Study of Amyloid Nephrosis, Arch. Dis. Childhood **2**:220 (Aug.) 1927.

9. Ophüls, W.: Nephritis: A New Series of Cases with a Review of Recent Literature, J. A. M. A. **65**:1719 (Nov. 13) 1915.

10. McElroy, J. B., in Tice, Frederick: Practice of Medicine, Hagerstown, Md., W. F. Prior Company, Inc., 1927, vol. 6, p. 599.

11. Osler, William, and McCrae, T.: The Principles and Practice of Medicine, ed. 2, New York, D. Appleton & Company, 1918, p. 711.

12. Osler, William, and McCrae, T.: The Principles and Practice of Medicine, ed. 10, New York, D. Appleton & Company, 1926, p. 717.

13. MacCallum, W. G.: Text-Book of Pathology, ed. 3, Philadelphia, W. B. Saunders Company, 1924, p. 302.

kidney may be the most prominent feature. Kaufmann [14] concurred in this opinion, but thought that the process is related to parenchymatous nephritis. The opinions of Frothingham [15] and Richardson [16] also fell in with this conception.

Amyloid has been produced experimentally by many workers. The most constant results are probably obtained by the use of staphylococci, but many other agents both chemical and bacterial have been used. Kuczynski [17] successfully produced the substance in animals by the continuous feeding of nutrose and cheese, and these experiments were repeated successfully by Smetana.[18] Frank [19] produced amyloidosis in animals by the use of the Friedländer bacillus. Many other organisms or their toxins have been used in the experimental production of amyloid, but from a clinical standpoint it is fairly well established that amyloid is most frequently associated with chronic tuberculous lesions, chronic suppuration of bone, syphilis, malignant tumors and leukemia. Wegelin [5] also mentioned its association with Hodgkin's disease.

The exact chemistry of amyloid is not yet thoroughly understood, but the generally accepted view is that advanced by Wells,[20] who believed that amyloid is a combination of chondroitin-sulphuric acid and a protein molecule. He pointed out that there is a high condroitin-sulphuric acid content in bone and in the lungs, and that in destructive lesions of these tissues there is undoubtedly an excess of this substance in the serum, which is carried to various organs, chiefly the liver, spleen, kidney and heart, where it is combined with the protein molecule. He thought that this substance is carried to the various organs in the serum rather than by the leukocytes, and this idea is supported by the fact that amyloid is never found within the epithelial or connective tissue cells. An entirely different theory was advanced by Smetana,[21] who thought that the deposition of this material has to do with a toxic damage to the reticulo-endothelial system, and he attempted to prove this theory by the injection of india ink into animals in which amyloid had already been produced. In these animals, he found that the

14. Kaufmann, E.: Spezielle pathologische Anatomie, ed. 2, Berlin, Georg Reimer, 1911, vol. 2, p. 844.

15. Frothingham, C.: Nelson's Loose Leaf Living Medicine, ed. 2, New York, Thomas Nelson & Sons, 1927, vol. 4, p. 701.

16. Richardson, H. B., in Cecil, R. L., and Kennedy, Foster: A Text-Book of Medicine, Philadelphia, W. B. Saunders Company, 1927, p. 904.

17. Kuczynski, M. H.: Virchows Arch. f. path. Anat. **239**:185, 1922.

18. Smetana, H.: Bull. Johns Hopkins Hosp. **37**:383, 1925.

19. Frank, A.: Beitr. z. path. Anat. u. z. allg. Path. **67**:181, 1920.

20. Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1914, p. 378.

21. Smetana, H.: Proc. Soc. Exper. Biol. & Med. **24**:187, 1926.

reticular cells did not phagocytose the india ink, whereas in normal animals these cells phagocytosed the ink particles readily. He interpreted this as being indicative of damage to, or abnormal function of, the reticulo-endothelial system.

There can be no question that amyloid is deposited in small quantities in many forms of nephritis, but to confuse these minimal deposits with the massive lesions of the kidney seen in general amyloidosis does not seem justifiable. Kumpf,[22] in a study of fifty cases of general amyloidosis, found practically no evidence of changes in the kidneys other than those produced by a simple deposit of amyloid, and it would seem that the cases of amyloidosis showing evidence of renal insufficiency should be considered, not as cases of nephritis, but rather as cases of a systemic disease in which the kidney has been damaged to such an extent that it may be the primary cause of death, or at least that it may produce the outstanding clinical symptoms.

We are reporting here three cases of general amyloidosis in which renal insufficiency was proved by clinical, chemical and pathologic methods. These cases are reported because this possible termination of amyloid disease seems to be little recognized in this country by either pathologists or clinicians. These cases represent the only proved instances of renal insufficiency in amyloid disease in a series of 11,000 necropsies recorded in the department of pathology at the University of Minnesota.

REPORT OF CASES

CASE 1.—A man, aged 27, was admitted to the Ancker Hospital on Sept. 29, 1928, complaining of pain and swelling of the left thigh. He stated that for several days before admission he had been riding on freight trains and that the jolting had aggravated an old draining sinus in the region of the hip. He was admitted to the surgical service, and physical examination showed numerous scars over the long bones and an old draining sinus of the left thigh. The thigh was swollen, red and tender, and at one point there was fluctuation. He had a high fever and a leukocytosis of 40,000. The thigh was incised and a soft tissue abscess was found, arising from a chronic osteomyelitis of the femur. After a few days, the fever subsided and the acute condition of the thigh improved. The patient was transferred to the medical service because the urine was found to contain large amounts of albumin, hyaline casts, leukocytes and erythrocytes. After this observation, the patient was questioned more closely and he stated that for eleven years he had had attacks of acute osteomyelitis involving various long bones, and that he had been told some years before by a physician that he had "Bright's disease." It was then noted that he had a moderate pitting edema of the ankles, which persisted throughout his entire stay at the hospital, but did not increase. The highest measurement of the systolic blood pressure was 105 mm. of mercury, and late in the patient's illness this dropped to 93. An examination of the eyegrounds, November 18, showed normal fundi. A roentgen examination of

22. Kumpf, A.: Unpublished thesis, Department of Pathology, University of Minnesota, 1929.

the chest showed no evidence of cardiac hypertrophy. Between October 1 and the time of the patient's death, December 16, eighteen urinalyses were done. Albumin was present in amounts varying from one to four plus. The specific gravity ranged between 1.010 and 1.028; hyaline and granular casts, leukocytes and a few erythrocytes were persistently present. Two concentration tests were done to determine the functional ability of the kidneys by combining the Mosenthal and the Volhard water tests. On one occasion, November 5, the range of the specific gravity was found to be between 1.008 and 1.010, and a similar test on November 13 showed a range of from 1.011 to 1.014. Three phenolsulphonphthalein tests, November 2, 9 and 11, showed no elimination of the dye. On four occasions between October 29 and December 6, the blood was examined for urea and creatinine. The readings ranged between 60 and 68.6 mg. of urea nitrogen and between 3.1 and 4.1 mg. of creatinine per hundred cubic centimeters of blood. The patient also showed a rapidly progressive secondary anemia. On admission, the erythrocyte count was 4,210,000 and the hemoglobin content 72 per cent. On December 3, the erythrocyte count was 2,800,000 and the hemoglobin content 56 per cent. In spite of the severe degree of the renal damage, the patient was fairly comfortable until a few days before his death. On December 16, he became stuporous and finally lapsed into coma and died.

From these observations a clinical diagnosis of chronic glomerulonephritis was made, although the low blood pressure and lack of cardiac hypertrophy could not be fitted into the picture.

The necropsy was performed on Dec. 16, 1928. External examination of the body showed a fairly marked degree of emaciation. There was a definite pitting edema of the ankles, and just below the inguinal ligament of the left thigh there was an old surgical incision, measuring 8 cm. in length. This wound was only partially healed, but showed no purulent drainage. There were scars over both greater trochanters, the outer aspect of the left tibia and the lateral surface of the left humerus, as well as over the eighth rib on the right side. These were apparently the result of multiple suppurative lesions of bones. The peritoneal cavity contained 2,000 cc. of a clear serous fluid, and the right pleural cavity contained 1,000 cc. of a similar fluid. The heart weighed 240 Gm., and showed an acute rheumatic endocarditis of the mitral leaflets, and adhesions between the aortic leaflets. The lungs were edematous and showed areas of bronchopneumonia along the posterior borders. The liver weighed 1,820 Gm., but showed no gross evidence of amyloid. The spleen weighed 260 Gm. The organ was firm and the capsule was tense, and, after cutting, the surface was found to be glassy and the corpuscles were not visible. The kidneys weighed 285 and 290 Gm. The capsules stripped easily, and the surfaces of the kidneys were pale and mottled by dark red areas. After the kidneys were cut, the cortices were found to be wide and the markings indistinct. The cortex and the medulla were well defined, the cortex being pale and the medulla dark red. The other organs showed little of interest. The spleen, liver and kidneys contained large amounts of amyloid, as demonstrated by the methyl violet stain. The microscopic picture in the kidneys will be discussed with the observations in the other cases.

CASE 2.—A woman, aged 35, had been married sixteen years and had never been pregnant. She gave a history of many previous infections. In childhood, she had had measles, scarlet fever, variola, pneumonia and "rheumatism." In 1912, she had had a salpingectomy and oophorectomy, and in 1913, an appendectomy. In 1917, osteomyelitis of the left tibia had developed and a curettement of the bone had been done. She was first admitted to the Ancker Hospital in December, 1921. At that time, she stated that she had syphilis and had received

a small amount of treatment in the form of mercury and arsenic. She also described an attack of constricting pain in the abdomen and chest seven years before, which the clinician interpreted as a gastric crisis. The Wassermann reaction of the blood was positive. Her chief complaint at the time of this admission was pain in the chest. A diagnosis of pleurisy and syphilis was made. Urinalysis showed a specific gravity of 1.032, a heavy trace of albumin and a few hyaline casts. She left the hospital against advice, but was again admitted in January, 1923, complaining of generalized abdominal pain. The urine at this time showed a specific gravity of 1.026, four plus albumin, hyaline casts, red blood cells and leukocytes. At this time, she was suspected of having gallbladder disease, but because of the urinary conditions surgical intervention was not deemed advisable. In 1925, however, at a private hospital, she was operated on and an empyema of the gallbladder was drained. Her third admission to the Ancker Hospital was on Nov. 21, 1927. Her complaints then were frontal headache and dyspnea. The headaches had been present for the year prior to her admission and were frequently associated with nausea and vomiting. She also complained of frequency of urination and blurring of vision. Her face was puffy, but there was no edema of the ankles. There was clinical and roentgen evidence of a slight cardiac hypertrophy. The blood pressure on two occasions was 175 systolic and 100 diastolic and 176 systolic and 104 diastolic. The urine showed albumin, casts, leukocytes and red cells. An examination of the blood on November 26 showed 47.6 mg. of urea nitrogen and 2.5 mg. of creatinine per hundred cubic centimeters. The patient also had a severe secondary anemia, the red cell count being 2,480,000 and the hemoglobin content 39 per cent. She again left the hospital against advice, but was readmitted on Dec. 3, 1927. At this time, she was markedly dyspneic, her face was puffy and there was a moderate edema of the ankles. Ophthalmoscopic examination showed albuminuric retinitis. The liver was palpable. Three readings of the blood pressure were 165 systolic and 95 diastolic; 180 systolic and 100 diastolic and 160 systolic and 90 diastolic. The average daily urinary output while the patient was in the hospital was 1,067 cc. Eleven urinalyses showed a specific gravity ranging from 1.014 to 1.024, with albumin varying from one to four plus. Casts, leukocytes and red cells were almost constantly present. Two examinations of the blood showed 116.9 mg. of urea nitrogen and 3 mg. of creatinine per hundred cubic centimeters and 147 mg. of urea nitrogen and 3 mg. of creatinine. The secondary anemia was even more pronounced, the hemoglobin content being 32 per cent and the erythrocytes 2,110,000. The patient gradually lapsed into coma and died on Jan. 10, 1928.

The necropsy was done on Jan. 10, 1928. External examination of the body showed it to be poorly nourished. There was a slight edema of the ankles. There was a midline scar in the lower part of the abdomen, and a second scar in the right upper quadrant of the abdomen. There was a scar over the anterior surface of the left tibia, and the bone was thickened beneath the scar. The peritoneal cavity was filled with a yellow, purulent fluid, containing flecks of fibrin. The pus was uniformly distributed throughout the abdomen, and the coils of the intestine were sealed together. There was no evidence of a primary infectious process in the appendix. The fallopian tubes and ovaries were absent. The uterus was small and appeared normal. The gallbladder was embedded in adhesions, but showed no evidence of an acute infectious process. There was no evidence of gastric or intestinal ulceration, or of diverticulitis, to account for the peritonitis. The pericardial sac contained no free fluid, but the visceral and parietal surfaces were sealed together by a thick layer of fibrin. The heart weighed 355 Gm. The valves and coronary arteries appeared normal. The

myocardium was firm but unusually pale. There was no gross evidence of amyloid. About 100 cc. of serous fluid was present in the right pleural cavity. There was an extensive terminal bronchopneumonia. The liver weighed 1,750 Gm. The methyl violet stain showed it to contain a small amount of amyloid. The spleen, similarly stained, showed no amyloid. The kidneys weighed 125 and 135 Gm. The capsules were thick and edematous. They stripped easily, leaving a finely granular surface. After cutting, the surfaces of the kidneys showed slightly narrowed cortices. The cortical markings were not distinct. The cortex and the medulla, however, were clearly demarcated. There was nothing in the gross appearance of the kidneys to suggest amyloid. The other organs showed nothing of note.

CASE 3.—A man, aged 55, who was acutely ill at the time of his admission to the hospital on Dec. 14, 1928, stated that he had not been well for about eight months. He first noticed swelling of his ankles, which did not incapacitate him. This edema persisted for about three months; then it gradually disappeared. About that time he began to have attacks of vomiting, not associated with pain. These occurred at irregular intervals. At times, he had repeated attacks of vomiting, followed by intervals during which he was symptom-free. His complaint at the time of admission was pain in the left side of the chest. He stated that he had taken cold about a week before, and that he had first noticed the pain about five days prior to his admission. This pain was not constant and was aggravated by deep breathing and by motion. There was no history of tuberculosis, chronic suppuration or syphilis, which might have been interpreted as an etiologic factor in the production of amyloid. He said, however, that he had had rheumatic fever twelve or fourteen years before.

Physical examination showed the patient to be well nourished. He had a Kussmaul type of respiration, and there was moderate cyanosis of the face and fingertips. The ankles were moderately edematous. The heart was enlarged to the right and to the left, and there was a pericardial friction rub. There was dulness at the base of the left lung and a friction rub in the same area. The liver was palpable. The blood pressure was 104 systolic and 50 diastolic on one examination. Ophthalmoscopic examination showed a slight edema of the optic disks. There were no hemorrhages, areas of exudate or vascular changes such as are frequently associated with hypertension. On one occasion, the urine showed four plus albumin, hyaline, granular and waxy casts, and pus cells. Examination of the blood on the day of admission showed 134 mg. of urea nitrogen and 3.9 mg. of creatinine per hundred cubic centimeters. On the day of admission, the carbon dioxide tension was 16 per cent by volume, and on the following day it was 7 per cent by volume. This patient also had a severe anemia, the erythrocyte count being 2,360,000 and the hemoglobin content 50 per cent. Shortly after admission to the hospital, he became drowsy and then stuporous, and finally lapsed into deep coma, and died two days after admission. The clinical diagnosis was subacute glomerulonephritis, pericarditis and uremia.

The necrosy was done on Dec. 16, 1928. The body was found to be well nourished and there was no evidence of edema. The peritoneal cavity contained no excess of fluid. The left pleural cavity contained about 500 cc. of a seropurulent fluid, which was encapsulated in an area comprising the superior and anterior two thirds of the pleural cavity. The balance of the pleural cavity was obliterated by dense fibrous adhesions. The right pleural cavity contained an equal quantity of fluid of similar character. The fluid was encapsulated in the postero-inferior portion of the chest cavity. The visceral and parietal layers of the pericardium were adherent to each other by fibrinous adhesions, giving the

heart a shaggy appearance. The pericardial sac contained about 75 cc. of a purulent fluid. The heart weighed 480 Gm. but this weight included the fibrinous exudate. The weight of the heart itself was estimated as 380 Gm. The mitral leaflets were thickened and nodular, but there was no evidence of acute rheumatic vegetations. The lungs showed a moderate degree of atelectasis and an excess of fluid and blood, but no evidence of consolidation. The spleen weighed 200 Gm. The follicles were prominent, but the organ showed no gross evidence of amyloid. The liver was enlarged, and showed no gross evidence of amyloid. Methyl violet stains of liver showed no amyloid, but those of spleen revealed deposits of this substance about the central arteries. Each kidney weighed 140 Gm. The organs were firm. The capsules were removed without difficulty, leaving a finely granular surface. After cutting, the surface of the kidney showed a definitely narrowed cortex. The cortex and the medulla were well defined. The pelves and the ureters

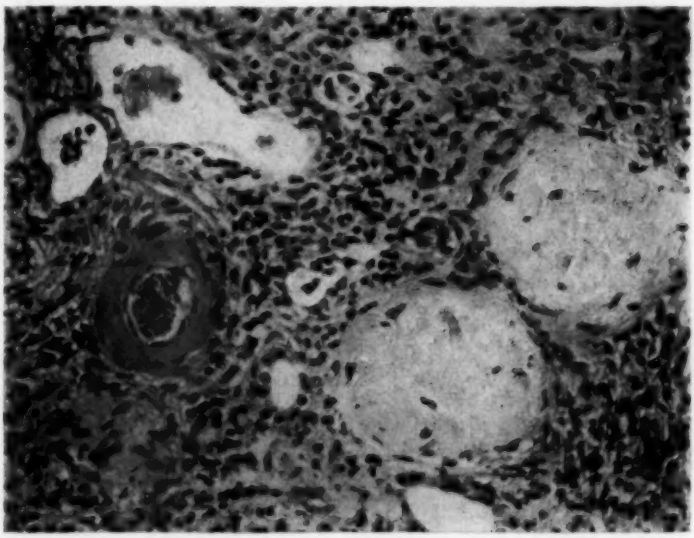

Fig. 1.—A section of amyloid kidney, showing thick-walled vessels and obliterated glomeruli. Hematoxylin and eosin stain.

were normal. Grossly, the kidneys could not be distinguished from kidneys showing a moderate degree of arteriolar involvement in a case of essential hypertension.

Microscopic Changes in the Kidneys in Cases 1, 2 and 3.—The kidneys in the three cases described were studied with hematoxylin and eosin, fat and amyloid stains. The microscopic material was fixed in a diluted solution of formaldehyde U. S. P. (1:10), and at the time of study some of the material had been in the fixing fluid fifteen or sixteen months. It was found that the microscopic pictures were materially improved if the sections were treated with ammonium hydroxide prior to staining, after the method of Davidoff. This improvement affected not only the hematoxylin and eosin stains, but also the amyloid stain. Instead of fixing in Zenker's solution, however, as suggested by Davidoff, the tissues for the amyloid stain were fixed in 70 per cent alcohol. This treatment of the tissue, it was found, restored the staining character of the amyloid so that the picture was as good as though the tissues had been fresh. The sections prepared in this way

and fixed in Zenker's solution showed poorer differentiation but more intense staining than those fixed in 70 per cent alcohol. In the preparations fixed in alcohol, the differentiation was distinct.

The microscopic pictures shown in the kidneys in these cases were so similar that one description will suffice for the three cases.

The outstanding deposits of amyloid were found in the glomeruli, and even in these cases representing the advanced stage of the disease there was a marked variation in the amount of amyloid in the individual glomerulus. However, even in those least involved, the capillaries were almost entirely occluded. As shown in figure 1, the glomeruli were a homogeneous mass of amyloid with complete occlusion of the capillary bed. According to Hueter,[23] the amyloid is laid down in the glomerulus between the basement membrane and the endothelium, gradually separating the endothelium from the basement membrane and eventually causing an atrophy of both. He also said that at times amyloid masses are found free in

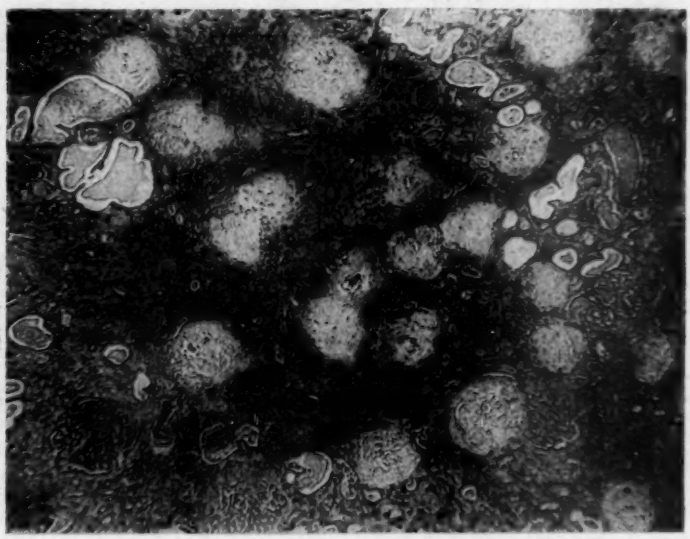

Fig. 2 (case 3).—A section of amyloid kidney, showing tubular atrophy and obliterated glomeruli. Hematoxylin and eosin stain.

the lumen of certain capillary loops. When the deposit of amyloid is so massive, however, the exact mechanism of deposition is not clear. The result, however, is an obliteration of the anatomic structure of the glomerulus with, necessarily, a complete cessation of function. Amyloid was also seen in the walls of many vessels. The larger arteries were not affected, but the smaller vessels, from interlobular arteries down to the afferent arterioles of the glomerulus, frequently showed thickened walls and narrowed lumen due to the deposit of amyloid (fig. 2).

Secondary to this vascular change in the glomeruli and outside of them was a marked atrophy of the tubules. The degree of atrophy varied with the individual case. It was most marked in case 3 (fig. 2) and somewhat less pronounced in case 1. Where the atrophy was not far advanced, the glomeruli themselves were

23. Hueter, C.: Centralbl. f. allg. Path. u. path. Anat. **19**:961, 1908.

not so completely closed and the tubules were separated by loose connective tissue. Scattered throughout the atrophic areas were numerous dilated tubules lined with flat epithelium. Many of these tubules contained casts, and a few of the casts took the characteristic amyloid stain with methyl violet. There was a surprisingly large number of inflammatory cells in these kidneys. These cells were both polymorphonuclear and lymphocytic. They were scattered through the atrophic areas in the interstitial tissue, as well as in clumps in the dilated tubules. The inflammatory infiltration is shown in figure 1.

The fat stains showed lipoid material in moderate quantities in the renal epithelium, and a few droplets in the interstitial tissue. The quantity of fat was not as marked as one would expect from Fahr's description, but in these cases there was relatively little epithelium remaining to undergo fatty degeneration, and perhaps in the earlier stages it was to be found in larger amounts.

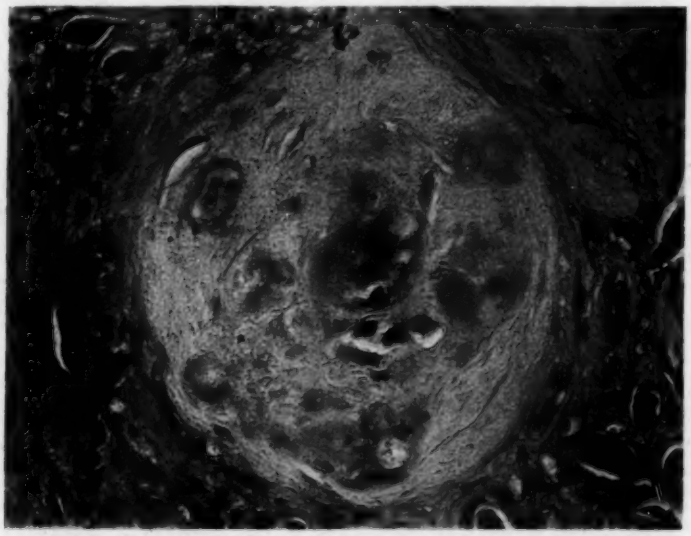

Fig. 3.—Section of amyloid kidney, showing a glomerulus with a transformation of amyloid to hyaline material. The dark stained masses in the center have taken the methyl violet stain. Amyloid stain was used.

In the glomeruli in which the amyloid was oldest, as indicated by the degree of obliteration of the glomerulus and the accompanying tubular atrophy, it underwent a change in its staining characteristics. It failed to take the methyl violet stain uniformly. There were blotchy areas (fig. 3) where the dark stained patches represented the amyloid that had taken the methyl violet stain, while the lighter stained material resembled hyaline. This fading seemed to occur from the periphery of the glomerulus inward, and in most of the glomeruli affected in this way a ring of unstained amyloid was seen in the periphery of the glomerulus. Fahr[7] mentioned the fact that in the far advanced stage, the amyloid is transformed into hyaline, and this is probably the change to which he refers. Wells also stated that, in the opinion of some workers, amyloid is first laid down as a hyaline substance, a precursor of amyloid, but he is of the opinion that this change in the staining character of the amyloid is degenerative, and in the cases studied

here it would appear that the change had occurred after the amyloid had been laid down in the usually recognized form. A similar change was seen in some of the amyloid deposited in the vessel walls.

There was no evidence in the sections to indicate that the kidneys had been involved by any disease process previous to the deposition of the amyloid. Two or three glomeruli showed a slight proliferation of Bowman's capsule, but in no instance was there any evidence of either a proliferative or an exudative inflammation of the glomeruli themselves, and the arterial changes were simply a deposit of amyloid within the vessel walls with no evidence of sclerosis of the usual type.

There were additional cases among our records which showed the same picture as that in the cases described, but the clinical data were too incomplete to prove renal insufficiency. In one of these cases there was an extensive arrested pulmonary tuberculosis. The kidneys were large and showed anatomic damage comparable to the changes in case 1 in the present series. The second case was well studied clinically. Apparently, the etiologic factor in this case was a multiple suppurative arthritis and sinusitis. Apparently, the patient died in uremia, but the last study of the blood, which was made a month prior to death, showed no retention of metabolites.

COMMENT

The pathologic process by which the kidneys in amyloid disease reach the state of renal insufficiency shown in these cases is primarily one of vascular damage affecting the glomeruli most severely, but also involving the arteries of small caliber. The condition of the glomeruli in the end-stage of an amyloid kidney is comparable to that seen in advanced glomerulonephritis, in that the capillary bed is occluded. In the case of glomerulonephritis, the capillary bed of the glomerulus is closed either by accumulation of leukocytes or by proliferative changes in the endothelium; whereas, in the amyloid kidney, the capillaries are occluded by a deposit of amyloid under the basement membrane. The effect on the tubules is the same in either case. When the blood supply in the glomerulus is shut off, there is a corresponding atrophy of the associated tubule. The afferent arteries to many of the glomeruli show thickened walls and narrowed lumina owing to the amyloid deposit. In no case was a complete occlusion of an afferent artery observed, and, while in many instances there must have been some reduction in the amount of blood reaching the glomerulus, the damage was primarily glomerular.

It is interesting to note that in case 1, as well as in one of the cases mentioned but not reported in detail, the kidneys were unusually large and showed no evidence of contraction. In all the references to this condition that we were able to find in the literature, the kidneys are described as being shrunken, but apparently the kidney can be damaged sufficiently by the deposit of amyloid to cause renal insufficiency before any contraction occurs. This state of affairs may be comparable to the closure of glomerular capillaries in a subacute glomerulonephritis

in which there is a retention of metabolites, with death from uremia while the kidneys are normal or even slightly larger than normal in size. The deposition of amyloid is usually thought of as a rather slow process, but, in some cases, at least, it is apparently sufficiently rapid to cause capillary closure and death before contraction can occur.

According to Kaufmann,[14] Volhard and Fahr[24] and others, hypertension is unusual in this type of disease, and only one of the three cases here described shows an elevation of blood pressure. The

Analysis of Three Cases of Amyloid Disease Involving the Kidneys

Examinations	Case 1	Case 2	Case 3
Age	27 years	35 years	55 years
Sex	Male	Female	Male
Etiology	Chronic osteomyelitis	Syphilis; chronic osteomyelitis	?
Chief complaint	Abscess of thigh	Impaired vision	Pleuritic pain
Duration of renal symptoms	?	6 years	8 months
Edema	Edema of ankles	Edema of face and ankles	Edema of ankles
Blood pressure	93 to 105 systolic	165 systolic and 95 diastolic to 190 systolic and 110 diastolic	104 sytolic and 50 diastolic
Eyegrounds	No examination	Albuminuric retinitis	Slight edema
Kidney function:			
Phenolsulphonphthalein	No excretion in three tests	No examination	No examination
Concentration	1,008 to 1,010; 1,011 to 1,014	No examination	No examination
Blood chemistry:			
Urea	60 to 68.6 mg.	47.6 to 147 mg.	134 mg.
Creatinine	3.1 to 4.1 mg.	2.5 to 3 mg.	3.9 mg.
Van Slyke	No examination	No examination	16 per cent to 7 per cent by volume
Urine:			
Specific gravity	1.010 to 1.028	1.012 to 1.032	?
Albumin	1 to 4 plus	Trace to 4 plus	4 plus
Microscopic observations	Hyaline and granular casts; white cells	Hyaline casts; white cells; red cells	Waxy casts
Blood:			
Hemoglobin content	56 to 72 per cent	32 to 55 per cent	50 per cent
Erythrocytes	4,000,000 to 2,800,000	3,660,000 to 2,110,000	2,360,000
Leukocytes	14,000 to 40,000	7,100 to 12,300	13,100
Heart weight	220 Gm.	355 Gm.	480 Gm.
Kidney weights	285 and 290 Gm.	125 and 130 Gm.	140 Gm. each

explanation for the lack of hypertension as a constant observation in these cases is not clear.

In the accompanying table, it can be seen that the clinical diagnosis of this condition must be difficult. Frothingham, and Osler and McCrae stated that the diagnosis cannot be made except in cases in which amyloidosis is suspected. Save for the absence of hypertension, a diagnosis of chronic glomerulonephritis is justifiable, and in case 2 with hypertension this diagnosis would be as close as possible. In

24. Volhard, F., and Fahr, Theodore: Die brightsche Nierenkrankheit, Berlin, Julius Springer, 1914.

cases 1 and 2, a clinical diagnosis of chronic glomerulonephritis was made, while in case 3 the diagnosis was subacute glomerulonephritis. From a study of these cases, however, it would seem well to bear in mind the condition of amyloid kidney in the differential diagnosis of chronic nephritis. In the absence of hypertension, when the history reveals any reason to suspect amyloidosis, a clinical diagnosis of amyloid kidney with renal insufficiency would seem justifiable.

CONCLUSIONS

Amyloid kidney is one manifestation of a systemic disease and not a specific form of nephritis.

It is primarily a vascular disease that involves predominantly the glomeruli.

It can lead to renal insufficiency, and when this occurs the kidney is usually contracted, but in some instances renal insufficiency occurs in kidneys of normal size or even in enlarged kidneys.

The clinical symptoms and signs of an advanced amyloid kidney resemble those of chronic glomerulonephritis save for the fact that in amyloid kidney hypertension is the exception rather than the rule.

Amyloid, after being deposited in the kidney, may undergo some change which alters its staining characteristics.

EXPERIMENTAL INFESTATION OF WHITE RATS WITH CYSTICERCUS FASCIOLARIS

MICROSCOPIC CHANGES IN LIVER, KIDNEY AND SPLEEN *

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The present paper is the result of a study undertaken to see what differences in cellular structure occur in the liver, kidney and spleen of the white rat as a result of an infestation with *Cysticercus fasciolaris*, the larval stage of the cat tapeworm, *Taenia crassicollis*. Under the proper conditions, it is possible experimentally to introduce large numbers of *Cysticercus* cysts into the liver of the rat. Miller and Dawley [1] (1928) studied the grosser physiologic effects of the parasite on the host and made a special study of the progressive changes in the blood following infestation. The formation of the cysts and the grosser microscopic changes in the liver accompanying the process were described by Bullock and Curtis [2] (1924) in their work on the production of *Cysticercus* sarcoma. In the present study of the effects of *C. fasciolaris*, particular attention has been paid to the chondriosomes of the hepatic and renal cells of the host. These important cell constituents have been found to vary in form, size and number in many different functional and pathologic conditions. No attempt will be made to review here the extensive literature on this subject. Cowdry [3] (1924) gave an adequate account of the form and function of the chondriosomes, while Findlay [4] (1927) had an extensive bibliography on their relations to cellular pathology. In the case of the spleen, the matter of chief interest was the cellular structure of the organ, and not the structure of the individual cells.

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* The work on which this paper is based was done in the Department of Zoology at Washington University, St. Louis, during the spring and summer of 1928.

1. Miller, H. M., and Dawley, C. W.: An Experimental Study of Some Effects of Cysticercus fasciolaris Rud. on the White Rat, J. Parasit. **15**:87, 1928.

2. Bullock, F. D., and Curtis, M. R.: A Study of the Reactions of the Tissues of the Rat's Liver to the Larvae of Taenia Crassicollis, and the Histogenesis of Cysticercus Sarcoma, J. Cancer Research **8**:446, 1924.

3. Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924.

4. Findlay, G. M.: Mitochondria and Cell Injury, J. Roy. Micr. Soc. **47**:258, 1927.

MATERIALS AND METHODS

The rats used were of the pedigreed Wistar stock raised in this department. The method of infestation was that used by Miller and Dawley.[4] Mature proglottids of adult worms taken from the intestines of cats were teased in a physiologic solution of sodium chloride and the oncospheres fed to the rats, either by being mixed with their food or by means of a medicine dropper. The infested animals and the controls were kept under as nearly the same conditions as possible; in some cases, infested rats and their controls were kept in the same cage. The latter were always killed at the same time as the animals known to be infested.

All rats were killed either by cutting off the head with large scissors or by crushing the head with one blow of a hammer. Each rat was opened as quickly as possible after killing and small pieces of tissue were cut out with a razor and placed immediately in the fixing fluid. The same order of removal was practiced in all cases, namely: liver first, kidney next and spleen last.

The fixatives used were Champy's chromo-osmic fluid and Regaud's mixture of potassium dichromate and formaldehyde for preservation of chondriosomes, and Bouin's solution of picric acid and formaldehyde for ordinary cell structure. Paraffin sections, from 4 to 5 microns thick, or material fixed in Champy's and Regaud's fluids were stained with the original Altmann stain (anilin acid fuchsin-picric acid) or with the Bensley-Cowdry modification of this (anilin acid fuchsin-methyl green). Sections, from 6 to 8 microns thick, of material fixed in Bouin's solution were stained with Ehrlich's hematoxylin and eosin, Haidenhain's iron hematoxylin and acid fuchsin, and with Mallory's triple connective tissue stain. Of the two fixatives used for the preservation of chondriosomes, Regaud's mixture gave the clearer fixation, but Champy's solution, while it did not penetrate as well, showed the pathologic changes in the liver to best advantage, and at the same time served for the study of the fat content. The Mann-Kopsch and Kolatchev methods for the demonstration of the Golgi apparatus were uniformly unsuccessful. The arsenious acid-silver nitrate method of Golgi was successful in only two cases, and the results were mediocre. No differences worthy of note could be distinguished.

MICROSCOPIC CHANGES IN THE LIVER PRODUCED BY C. FASCIOLARIS

Conditions in Normally Functioning Liver Cells.—Before describing the differences between the liver cells of normal and those of infested rats, it will be necessary to make a few statements with regard to the chondriosomal content of the normal liver cell. Rathery[5] (1909) described the liver cell of the rabbit after using a chromo-osmic fixative. He found the chondriosomes in the granular form, mitochondria. Two types of cells were differentiated: one in which the mitochondria are scattered fairly uniformly in a homogeneous cytoplasm, which he called the "granular state"; and another, in which large clear spaces are found, forcing the cytoplasm which contains the mitochondria to the sides of the cell and around the nucleus and in between the spaces, the "clear state." The former he considered the normal condition. On the other hand, Nöel[6] (1923), using Regaud's potassium dichromate-formaldehyde

5. Rathery, F.: La cellule hépatique normale, Arch. de méd. expér. et d'anat. path. **21**:50, 1909.

6. Noël, R.: Recherches histophysiologiques sur le cellule hépatique des Mammifères, Arch. d'anat. micr. **19**:1, 1923.

fixative, found the chondriosomes of the liver cells of the rat in the form of both granules and rods, or chondriocontes. He believed the clear state to be the normal state. The results of the present study confirm those of the authors mentioned, in that chondriosomes are found to be granular after chromo-osmic fixation, and both granular and rod-shaped after potassium dichromate-formaldehyde fixation (figs. 1 and 3). Cells of both clear and granular type are found. Both may be present in the liver of the same animal, either in approximately equal numbers or with one type predominating. In other cases, only one type of cell is found. This variation cannot be correlated with differences in fixation, as postulated by Rathery, nor with differences in the nutritional state of the animals, since both types seem to be normal.

In the present study, actively functioning hepatic cells of rats heavily infested with *C. fasciolaris* showed certain chondriosomal variations from the normal. There was a definite increase in the size of the chondriosomes in cells of infested animals, noticeable after both types of fixation (figs. 1 and 2; 3 and 4). In cells fixed with Regaud's potassium dichromate-formaldehyde, it was seen that a decrease in the number of chondriocontes had occurred as a result of the infestation (figs. 3 and 4). An attempt was made to count the chondriosomes in several cells of each liver in order to determine whether there had been an increase in the number of chondriosomes in the cells of the infested animals. Owing to certain technical difficulties, no numerical results are offered, but the general statement can be made that there is an increase in the number of chondriosomes per cell in the liver of an infested rat. These results point to an increased activity in the functioning liver cells of infested rats. The increased size of the chondriosomes, the change from chondriocontes to mitochondria and the increased number of chondriosomes are all well known evidences for an increased cell activity. The explanation is obvious; the destruction of large amounts of liver tissue by the growth of the cysts throws an increased burden on the remaining cells with a corresponding necessity for hyperfunction.

Changes in Hepatic Cells under Pathologic Conditions.—Bullock and Curtis [2] called attention to three pathologic conditions in the livers of rats infested with *C. fasciolaris:* (1) necrosis of liver cells, (2) pressure atrophy and (3) fatty infiltration. These have been investigated in the present study by the use of chondriosomal fixation and the staining of liver tissue from heavily infested rats. In addition, a fourth condition has been observed, which is assumed to be that of cellular hyperfunction.

As stated by Bullock and Curtis, necrosis occurs mainly during the early stages of the formation of the cyst, and to any great extent

only in a few cases. In the present work, the shortest time elapsing between infestation and the taking of tissue was nineteen days. In one of these cases, a slight necrosis was still visible, mainly in liver cells that had been included in the cyst wall, although a few such cells were also found at the edge of the cyst. These cells were vacuolar (fig. 6); the size of the vacuoles ranged from about that of a mitochondrium to about twice that size. It was evident that the vacuoles were the remains of mitochondria, for every gradation from normal mitochondria to full-sized vacuoles could be found. The former seemed to swell and gradually to lose their staining power, a few at a time, until the whole cell was full of vacuoles. Presumably, the cell then disintegrated. So far as is known, there has been no similar observation on the relation of mitochondria to the vacuoles of degeneration. Lewis [7] (1919) observed vacuolar degeneration in cells in tissue cultures, but the mitochondria did not seem to be involved. The results of the present study of cells in necrosis are also different from those obtained by Mayer, Rathery and Schaeffer [8] (1909, 1910) in their study on the autolysis of liver cells of the rabbit. These differences are probably to be explained on the basis that the mechanism of cell destruction in the one case is entirely different from that in the other.

The changes in the chondriosomes of cells undergoing pressure atrophy were slight. Such cells, found at the edges of the cysts, showed only those changes that have been described for normally functioning liver cells of infested animals; i.e., an increase in the number and the size of the chondriosomes and a tendency for the chondriocontes to give way to mitochondria.

In the livers of rats killed during the earlier stages of the infestation (at from nineteen to thirty days), the cysts were surrounded each by a more or less broad band of cells in which the mitochondria were enormously swollen, but retained their staining properties. In some cases, the mitochondria were even more deeply stained than normally. This resulted in a condition in which the cells were practically filled with large, brightly staining, red globules (fig. 5). There was a gradual transition from these cells to the normally functioning cells at the edge of the zone. So far as could be observed, there was no fusion of the globules such as that in autolysis as described by Mayer, Rathery and Schaeffer, nor did the cells tend to disintegrate, and the condition disappeared as the infestation proceeded. A similar condition in which

7. Lewis, M. R.: Degeneration Granules and Vacuoles in the Fibroblasts of Chick Embryos Cultivated in Vitro, Bull. Johns Hopkins Hosp. **30**:81, 1919.

8. Mayer, A.; Rathery, F., and Schaeffer, G.: Lésions expérimentales des cellules du foie, Compt. rend. Soc. de biol. **67**:709, 1909; Lésions expérimentales de la cellule hépatique, Arch. de méd. expér. et d'anat. path. **22**:177, 1910.

the globules fused in autolysis of liver cells, was described by Dyson [9] (1912). However, the evidence seems to show that these cells do not go to pieces, but return to the normal condition after the infestation has proceeded for a time. The assumption has been made that this is a condition of intense hyperfunction on the part of cells surrounding the places where the greatest amount of tissue has been lost. Another explanation is suggested by the arrangement of these cells in a band about the cyst, namely, that a toxin is produced by the parasite, which diffuses out of the cyst and affects the cells, causing them to undergo this change. Miller and Dawley could not demonstrate any toxicity of the cyst fluid, nor has any such toxin ever been found or its effects on cells described. However, this is also a possible explanation.

The present study confirms the previous observation of Bullock and Curtis that a moderate fatty infiltration of the liver occurs in the earlier stages of the infestation. The globules of fat occurred in largest numbers around the central veins of the lobules and around the cysts. They were also numerous in the inner layer of the cyst wall. These droplets of fat occurred both in the sinusoids and in the liver cells themselves. In the latter case, an interesting condition was observed (fig. 7). The cells were normal with one exception: the nuclei stained as usual, and the chondriosomes stained with the same intensity as those of normally functioning cells; but with the appearance of fat in the cells, there was a corresponding decrease in the number of chondriosomes. There can be no doubt that the droplets were true fat and not lipoids. Both fats and lipoids were blackened by the osmic acid during the fixation with Champy's fluid, but, in the subsequent treatment of sections with 1 per cent potassium permanganate, the lipoids were decolorized immediately, while the fat was decolorized only after treatment for from ten to fifteen minutes followed by alcohol and xylene.

The decrease in the number of chondriosomes accompanying the appearance of fat in the cells raises the question as to whether there is a true fatty infiltration, or whether it may not be a fatty degeneration. The immense literature on the subjects of fatty infiltration and fatty degeneration has been adequately reviewed by Wells [10] (1925) and will not be reviewed here. The chief evidence for a fatty degeneration given by the present study was the disappearance of chondriosomes incidental to the appearance of fat. Several authors, notably Scott [11]

9. Dyson, W.: The Staining of Granules in the Liver Cells Before and After Autolysis, J. Path. & Bact. **17**:12, 1912.

10. Wells, H. G.: Chemical Pathology, Philadelphia, W. B. Saunders Company, 1925.

11. Scott, W. J.: Experimental Mitochondrial Changes in the Pancreas in Phosphorus Poisoning, Am. J. Anat. **20**:237, 1916.

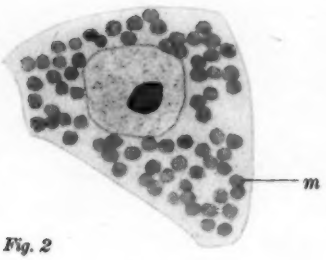

Fig. 2

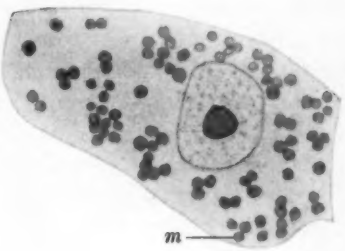

Fig. 1

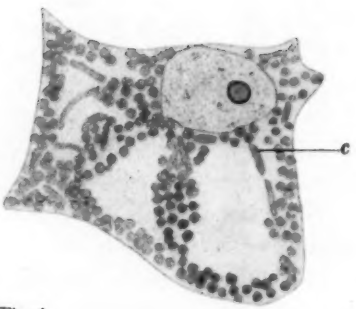

Fig. 4

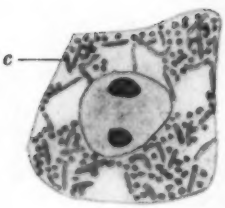

Fig. 3

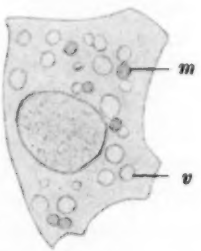

Fig. 6

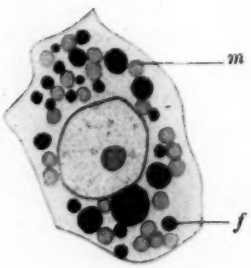

Fig. 7

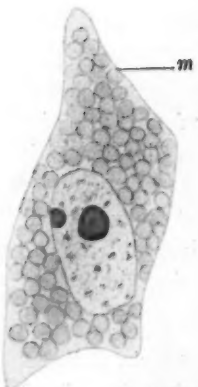

Fig. 5

Explanation of Plate

All figures were drawn with the aid of a camera lucida, using a Bausch and Lomb oil immersion lens and 10× ocular. The original drawings were enlarged by one-fourth with the pantograph and were reduced to the original size in printing. Total magnification, × 1,250.

Abbreviations: *c*, chondriosomes; *f*, fat globules; *m*, mitochondria; *v*, vacuoles.

Fig. 1.—A normal liver cell in the granular state, of an uninfested rat weighing 205 Gm. The liver weighed 9 Gm. Champy fixation; stained with acid fuchsin-methyl green.

Fig. 2.—A normal liver cell in the granular state, of an infested rat weighing 190 Gm. The liver weighed 32 Gm. The cell shown is one taken nineteen days after the infestation of the rat. Champy fixation, stained with acid fuchsin-methyl green.

Fig. 3.—A normal liver cell in the clear state, of an uninfested rat weighing 145 Gm. The liver weighed 6 Gm. Regaud fixation, acid fuchsin-methyl green stain.

Fig. 4.—A normal liver cell in the clear state, of an infested rat weighing 272 Gm. The liver weighed 88 Gm. The cell shown was taken fifty-two days after infestation. Regaud fixation, acid fuchsin-methyl green stain. (Figures 3 and 4 are of the same magnification; it may be seen here that the chondriosomes in figure 4 are actually, as well as seemingly, larger than those in figure 3.)

Fig. 5.—An abnormal liver cell of the same rat as the cell in figure 2. Champy fixation, acid fuchsin-methyl green stain.

Fig. 6.—A vacuolar liver cell of the same rat as the cells in figures 2 and 5. Champy fixation, acid fuchsin-methyl green stain.

Fig. 7.—A liver cell with fat globules, of an infested rat weighing 140 Gm. The liver weighed 30 Gm. The cell was taken nineteen days after infestation. Champy fixation, acid fuchsin-picric acid stain.

(1916) and Noël[6] (1923), described the formation of fats from the chondriosomes, while other investigators, Hess and Saxl[12] (1910), Ignatowitsch[13] (1914) and Fischer and Hooker[14] (1917), stated their belief that fat is formed from the masked lipoids of the cell, which are probably the chondriosomes. On the other hand, the work of several others may be cited in favor of the view that this is a case of fatty infiltration. According to Lewis[15] (1918), when cells in tissue cultures form fat droplets, the chondriosomes are not involved. Munk[16] (1908) and Krontowski and Poleff[17] (1914) showed that fat may be formed in cells or taken up by them from the surrounding medium, and that the former process occurs only during autolysis and degeneration. In the present work, it was evident that the cells containing the fat were not degenerating, and so it would seem that these cells took up the fat from the sinusoids. It is probable that the disappearance of the mitochondria was due to the solvent action of the fat, since, as is known, the lipoids are fat-soluble. The author is therefore of the opinion that Bullock and Curtis were right in their description of the condition as a fatty infiltration.

As an explanation of the condition of fatty infiltration, two views may be presented. Fischer and Hooker[14] pointed out that fatty infiltration of the liver occurs as a result of an acidosis due to lowered oxidation. It seems likely that such a condition would occur in the livers of infested rats, since the presence of a large number of cysts occupying the end-branches of the portal veins (Bullock and Curtis) must lead to an interference with the circulation of the liver and result in a lowered oxidation of the cells. The other explanation is based on the work of Coope and Mottram[18] (1914), who found a fatty infiltration in the livers of female cats during pregnancy. Their explanation is that the liver, under the condition of heightened activity, can no longer metabolize the fat in the amounts supplied, so that it piles

12. Hess, Leo, and Saxl, P.: Eiweissabbau und Zellverfettung, Virchows Arch. f. path. Anat. **202**:148, 1910.

13. Ignatowitsch, D.: La dégénérescence graisseuse "in vitro," Compt. rend. Soc. de biol. **76**:607, 1914.

14. Fischer, M. H., and Hooker, M. O.: Fats and Fatty Degeneration, New York, John Wiley & Sons, 1917.

15. Lewis, M. R.: The Formation of the Fat Droplets in Cells of Tissue Cultures, Science **48**:398, 1918.

16. Munk, F.: Ueber lipoide Degeneration, Virchows Arch. f. path. Anat. **194**:527, 1908.

17. Krontowski, A., and Poleff, L.: Ueber das Auftreten von lipoiden Substanzen in den Gewebs-kulturen und bei der Autolyse der entsprechenden Geweben, Beitr. z. path. Anat. u. z. allg. Path. **58**:407, 1914.

18. Coope, R., and Mottram, V. H.: Fatty Acid Infiltration of the Liver During Pregnancy and Lactation, J. Physiol. **49**:23, 1914.

up in the sinusoids of the organ. This explanation is as feasible as the former one; the evidence for a heightened activity in the liver cells of infested rats has been presented earlier in this paper. It seems unnecessary to choose between these two explanations; both are equally applicable to the present case, and probably both operate together.

MICROSCOPIC CHANGES IN THE KIDNEY

No constant cytologic differences could be observed between the kidney cells of normal and those of infested rats. This is rather surprising when one considers the close relationship existing between liver function and kidney function. It is still more surprising in view of the fact that large amounts of liver tissue were being destroyed and the products taken up by the blood, and the fact of the extra load thrown on the kidneys by the waste products of the parasite in the liver. The fact that no visible changes were found speaks against the theory that a toxin is formed by the parasite. According to Simonin [19] (1920), during chronic toxemia from verminous toxins, chronic nephritis is present, and the cells of the convoluted tubules of the kidney become greatly vacuolar. No such conditions could be observed in the present study, and the negative result must be taken as further indication of the fact that *C. fasciolaris* is a benign parasite (Miller and Dawley).

CHANGES IN THE CELLULAR CONTENT OF THE SPLEEN DUE TO INFESTATION WITH C. FASCIOLARIS

Comparison of sections of the spleens of normal with those of infested rats showed that there had been a great increase in the number of megakaryocytes in the infested animals. These cells are easily recognizable by virtue of their large size, lobed nuclei and granular cytoplasm staining lavender with hematoxylin and eosin after Bouin's fixation. That megakaryocytes are found normally in small numbers in the spleen of the rat has been asserted by De Kervily [20] (1912), who stated that the normal number may be as high as thirty per square millimeter. An attempt was made to determine the relative increase in number by actual count, using the following method:

The unit of measure taken was the number of megakaryocytes per field. Counts were made with the high-power objective and 10 × ocular, which gave a field of about 0.44 mm. diameter by measurement with a stage micrometer. The total number of fields per section and the total number of megakaryocytes were recorded for three sections each of the spleens of six infested rats and two controls. The

19. Simonin, P.: Introduction à l'étude des toxins vermineuses, Nancy, Humbolt & Cie, 1920.

20. De Kervily, M.: Sur la présence de megakaryocytes dans la rate de plusieurs Mammifères adults normaux, Compt. rend. Soc. de biol. **72**:34, 1912.

sections were of material fixed in Bouin's solution; they were 8 microns thick and stained with Ehrlich's hematoxylin and eosin. When there was any doubt as to the identity of the cell, it was not counted, which may account for the rather low number found in normal rats (compare with figure given by De Kervily[20]). The results are set forth in the accompanying table.

It may be seen from the table that all three factors, namely, the number of cysts present, their size and the duration of the infestation, are of importance in determining the increase in the number of megakaryocytes in the spleen. However, these three factors are not of equal importance; the size of the cysts and the duration of the infestation seem more important than the number of the cysts. For example, rat 7, with 6,030 cysts of 1 mm. diameter, showed a much smaller increase than rat 9, with 2,720 cysts of 2 mm. diameter, although the length of infestation was the same. It is also of interest to compare rats 8 and 160, in which the cysts were large and the duration great,

Comparison of Normal and Infested Animals with Regard to the Number of Megakaryocytes in the Spleen

Rat	Cysts	Diameter of Cysts, Mm.	Length of Infestation, Days	Total Count of Megakaryocytes in 3 Sections	Number of Fields in 3 Sections	Ratio of Megakaryocytes to Fields
3	....	..	..	1	191	.005
1	....	..	..	4	191	.021
7	6030	1	19	19	138	.138
160	1	8	.. *	43	288	.149
11	2160	2	24	62	213	.291
9	2720	2	19	108	145	.745
8	116	3-7	52	300	373	.804
12	3025	2	14	227	278	.817

* Accidentally infested at some time previous to beginning experiment.

although the number of cysts was less, with the other rats of the series. However, the number of animals in the series was far too few to justify the drawing of any definite conclusions as to the relative importance of these three factors.

The significance of the increase in the number of megakaryocytes in the spleen of an infested rat is not clear. The function of the megakaryocytes was believed by Wright[21] (1906, 1910) to be the formation of thrombocytes, or blood platelets, and this work was confirmed by Bunting[22] (1909) and Downey[23] (1913). According to Bunting, a thrombocytosis is accompanied by an increase in the number of megakaryocytes in the bone-marrow. However, there is no evidence of a thrombocytosis in the case of infestations with *C. fasciolaris,* nor

21. Wright, J. H.: The Origin and Nature of the Blood Plates, Boston M. & S. J. **154**:643, 1906; The Histogenesis of the Blood Platelets, J. Morphol. **21**:263, 1910.

22. Bunting, C. H.: Blood Platelet and Megakaryocyte Reactions in the Rabbit, J. Exper. Med. **11**:541, 1909.

23. Downey, H.: The Origin of Blood Platelets, Folia Haemat. **15**:25, 1913.

could Miller and Dawley observe any significant difference in the clotting time of blood between infested rats and their controls. So far as can be determined from the literature, no observation similar to the present one has been made in the case of any other parasitic infestation. Donhauser [24] (1908) found megakaryocytes in the spleen of a patient with metaplasia, but no such condition is to be seen in the spleens of infested rats. It is of interest that Bunting [25] (1911) showed that the megakaryocytes increase in number in the bone-marrow of patients with Hodgkin's disease. However, the eosinophilia in Hodgkin's disease is local, and not general as shown for *C. fasciolaris* by Miller and Dawley. Such a parallelism is nevertheless not without obvious value in this case, for the etiology of Hodgkin's disease has never been satisfactorily determined. I believe that a study should be made of the megakaryocytes in a number of other parasitic diseases in order to determine whether this enormous increase is a general condition or one specific for the cysticercosis of the rat.

CONCLUSIONS

The liver cells of uninfested white rats may be in either the "clear" or "granular" state independent of the fixative used, and as far as can be ascertained, also independent of the nutritional state of the animal.

The chondriosomes of the liver cells of white rats may be in the form of granules (mitochondria) or of both granules and rods (chondriocontes), dependent on the type of fixative used; the former condition appears after fixation with chromo-osmic fluid, the latter after fixation with the potassium dichromate-formaldehyde mixture.

Functioning liver cells of white rats heavily infested with *Cysticercus fasciolaris* show the characteristic picture of increased activity, namely, an increase in the size and the number of the chondriosomes and a tendency for the chondriocontes to fragment into mitochondria.

In the necrosis of liver cells of infested rats occasionally accompanying the formation of the cysts, there is a gradual decrease in the number of mitochondria. These lose their staining properties and swell, becoming vacuoles, so that the entire cell just before disintegration is vacuolated.

Liver cells undergoing pressure atrophy show only those chondriosomal changes that are found in functioning liver cells of infested rats.

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24. Donhauser, J. L.: The Human Spleen as an Haematoplastic Organ, etc., J. Exper. Med. **10**:559, 1908.

25. Bunting, C. H.: Blood Platelets and Megakaryocytes in Hodgkin's Disease, Bull. Johns Hopkins Hosp. **22**:114, 1911.

In a more or less broad band of cells around each cyst in livers of infested rats there is observed a condition, with transition to the normally functioning cells, in which the mitochondria are intensely stained and extremely swollen so as to fill almost the entire cell. The assumption has been made that this is evidence of extreme hyperfunction, although it may possibly be evidence of the formation of a toxin in the cysts.

The observation of Bullock and Curtis that a fatty infiltration of the liver occurs in infested rats is confirmed by this work. The fat is found as globules around the centrolobular veins, around the cysts and in the inner layer of the cysts. Fat is found both in the sinusoids and in the cells, in the latter case with a corresponding decrease in the number of mitochondria, so that this condition might be considered as evidence for a fatty degeneration rather than a fatty infiltration. However, from the fact that these cells containing fat show no signs of degeneration, the conclusion is reached that the process is one of true infiltration, with taking up of fat by the cells with solution of the mitochondria in the fat. Two explanations are offered for the fatty infiltration, one on the basis of impeded circulation with a resulting lowered oxidation and acidosis, the other on the basis of heightened activity of the organ.

No constant cytologic changes could be observed in the kidney cells of infested rats, which opposes the theory of toxin formation.

In the spleens of infested rats there is observable an increase in the number of megakaryocytes per field of high power magnification. No explanation of the condition can be offered, but in this and in the presence of an eosinophilia, the cysticercosis of the rat resembles Hodgkin's disease in man.

TRAUMATIC PORENCEPHALY *

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CHICAGO

Porencephaly is usually defined as a funnel-shaped defect of the brain which extends from the surface of a hemisphere close to or into the lateral ventricle. The defect is covered by a membrane of varying thickness and is filled with a clear, colorless fluid; thus, on external examination, it appears as a cyst.

In the first descriptions, which date from more than a century ago,[1] these defects were considered as malformations, an explanation at first also given by Heschl,[2] who in 1859 introduced the term porencephaly. While Heschl later changed his explanation in favor of circulatory disturbances, particularly a lack in the blood supply of the involved regions of the brain, Schattenberg,[3] von Kahlden [4] and Ziehem maintained the dysontogenic origin. Heschl's vascular theory was taken over by Kundrat,[5] Bourneville and Schwartz [6] and many other investigators. A number of authors discussed the significance of traumatic and inflammatory changes.

The theory that has the greatest weight of evidence is undoubtedly that which emphasizes the importance of trauma. Defects of the brain similar to those which are apparently congenital may develop in later life from areas of traumatic, hemorrhagic softening (Boettger,[7] von

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* From the Department of Pathology of the Cook County Hospital.

1. The early literature on porencephaly is reviewed by Schuette, E.: Die pathologische Anatomie der Porencephalie, Centralbl. f. allg. Path. u. path. Anat. **13**:633, 1902.

2. Heschl, R.: Gehirndefect und Hydrocephalus, Vrtljschr. f. d. prakt. Heilk. **61**:61, 1859.

3. Schattenberg, A.: Ueber einen umfangreichen porencephalischen Defekt des Gehirnes bei einem Erwachsenen, Beitr. z. path. Anat. u. z. allg. Path. **5**:121, 1889.

4. Von Kahlden: Ueber Porencephalie, Beitr. z. path. Anat. u. z. allg. Path. **18**:2, 1895.

5. Kundrat, H.: Die Porencephalie. Eine anatomische Studie, Graz, 1882.

6. Bourneville and Schwartz: Nouvelle contribution à l'étude de la porencéphalie et pseudo-porencéphalie, Progrès méd. **8**:37, 1898.

7. Boettger, quoted from Schroer: Zur Kenntnis der traumatischen Porencephalie, Virchows Arch. f. path. Anat. **262**:144, 1926.

Kahlden, Kaufmann,[8] Kennard,[9] Kopp,[10] Landouzy and Labbé,[11] LeCount and Semmarek [12] (case II of their series), Ponfick,[13] Schroer,[14] Struchlik-Sirotow [15] and others). Von Kahlden distinguished these acquired forms from the true congenital porencephaly, a differentiation which had to be given up because it was impossible to draw a borderline between true and acquired porencephaly (Beyer [16]). Siegmund [17] called any defect of the brain from an aseptic necrosis porencephaly.

The trauma that leads to a porencephaly may affect the brain while the fetus still is in the uterus (Seitz,[18] Kikuth [19]) or it may occur during birth, in infancy or in later life. The most common cause is undoubtedly the trauma occurring at birth (Frangenheim,[20] d'Hollander and Schmidt,[21] Siegmund, Schwartz [22] and others). In the history of cases of porencephaly there is a striking frequency of prematurity, prolonged labor and instrumental delivery. The investigations of Siegmund and, in particular, of Schwartz have revealed a continuous chain of changes from the areas of loosening and softening of the brain substance and the hemorrhages as immediate sequels of the birth trauma to the fully developed porencephalic cysts. In addition to the direct action of the

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8. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin-Leipzig, Vereinigung Wirinschaftlicher Verleger 1922, vol. 2, p. 1441.

9. Kennard, K. S.: Case Report: Primary Tumor of the Heart. Porencephalus, New York State J. Med. **21**:346, 1921.

10. Kopp, J.: Ein Fall von Porencephalo-Hydrocephalia traumatica unilateralis permagna, Deutsche Ztschr. f. Chir. **116**:226, 1912.

11. Landouzy and Labbé, M.: Les porencéphalies traumatiques, Presse méd. **7**:66, 1899.

12. LeCount, E. R., and Semerak, S. B.: Porencephaly, Arch. Neurol. & Psychiat. **14**:365 (Sept.) 1925.

13. Ponfick, E.: Ueber Hirncysten und Porencephalie, Centralbl. f. allg. Path. u. path. Anat. **8**:858, 1897.

14. Schroer (footnote 7).

15. Struchlik-Sirotow, S.: Zur Frage über die sekundäre Degeneration der Pyramidenbahnen bei Porencephalie, Arch. f. Psychiat. **54**:1056, 1914.

16. Beyer, E.: Zur Lehre der Porencephalie, Neurol. Centralbl. **15**:823, 1896.

17. Siegmund, H.: Die Entstehung von Porencephalien und Sklerosen aus geburtstraumatischen Hirnschaedigungen, Virchows Arch. f. path. Anat. **241**:237, 1923.

18. Seitz, L.: Ueber die durch intra-uterine Gehirnhämorrhagien entstandenen Gehirndefekte und die Encephalitis congenita, Arch. f. Gynäk. **83**:701, 1907.

19. Kikuth, H.: Beitrag zur Genese und Klinik der Porencephalie, Jahrb. f. Kinderh. **111**:112, 1926.

20. Frangenheim, P.: Aetiologie und Behandlung der Cephalhydrocele traumatica, Arch. f. klin. Chir. **152**:676, 1928.

21. D'Hollander and de Schmidt, P.: Contribution à l'étude anatomo-clinique de la sclérose lobaire, Arch. internat. de méd. expér. **1**:1, 1924.

22. Schwartz, Philip: Die traumatischen Schädigungen des Zentralnervensystems durch die Geburt. Anatomische Untersuchungen, Ergebn. d. inn. Med. u. Kinderh. **31**:165, 1927.

trauma on the brain, circulatory disturbances in the large veins of the brain from a backing up of the blood in the sinuses of the dura mater are of great importance.

Several authors have tried to associate the porencephaly with inflammatory processes in the brain and leptomeningi (Babonneix,[23] Ball and Anger,[24] Globus,[25] Henkel,[26] LeCount and Semerak, Sironi,[27] Stenström,[28] Winterode and Lewis [29]). Syphilis has been blamed for it by Henkel, LeCount and Semerak (case III) and by Delore and Pic.[30] It has to be kept in mind, however, that the presence of inflammatory changes in a brain with a porencephalic defect does not necessarily mean that the inflammation has produced the defect. For instance, in the case of tuberculous porencephaly published by Winterode and Lewis, it is much more likely that the child had first porencephaly and later tuberculous encephalitis than that the latter caused the defect. It is well known that the cerebral lesions from birth trauma, glial scars and cysts remain points of diminished resistance in which at a later time complicating changes are apt to occur (Schwartz). These complicating changes account, at least in part, for the fact that the first clinical manifestations of the porencephaly may appear months or even years after birth or that the symptoms may be aggravated with progressing age.

The case of porencephaly that is the subject of this paper is of interest not only because the traumatic origin seems to be well proved, but also because microscopically the brain shows changes some of which are still a matter of discussion.

REPORT OF A CASE

Clinical History.—A white American woman, aged 22, was brought to the Cook County Hospital in such an exhausted condition that the history could not

23. Babonneix, L., and Lhermitte, J.: Étude histologique des plaques fibro-myéliniques du cortex cérébral et de la pia mère dans un cas de microcéphalie avec porencéphaly, Compt. rend Soc. de biol. **88**:1014, 1923.

24. Ball, V., and Anger, L.: Encéphalopathies atrophiques du jaune âge. Porencéphalie vraie unilatérale et idiotie chez un chat, J. de méd. vétérin. et de zootechn. **72**:397, 1926.

25. Globus, J. H.: A Contribution to the Histopathology of Porencephalus, Arch. Neurol. & Psychiat. **6**:652 (Dec.) 1921.

26. Henkel, K.: Polioencephalitis mit Ausgang in Porencephalie, Ann. d. Krankenhäuser zu München **96**:127, 1899.

27. Sironi, L.: Contributo clinico ed anatomo-pathologico allo studio delle eterotopie midollari, Riv. di clin. pediat. **19**:705, 1922.

28. Stenström, N.: Arachnoiditis hemorrhagica together with porencephalia, Acta med. Scandinav. **56**:591, 1922.

29. Winterode, R. P., and Lewis, N. D. C.: A Case of Porencephalic Defect Associated with Tuberculous Encephalitis, Arch. Neurol. & Psychiat. **10**:304 (Sept.) 1923.

30. Delore, M. P., and Pic, M.: Maladie polykystique du foie et des reins. Porencéphalie avec hémiplégie infantile, Lyon méd. **60**:489, 1928.

be taken. Every four or five minutes she had seizures which started with a tonic convulsion lasting for from five to ten seconds. During this convulsion, the head was drawn backward and the face was turned to the right. The right arm was extended, and the left arm was flexed. The legs were extended, and there was a plantar flexion of the feet. The tonic stage was followed by clonic convulsions, which involved the entire body, except the left side of the face, and lasted for about forty seconds. There was nystagmus with a quick component to the left. After the attacks, the eyes moved aimlessly and at times deviated laterally. The breathing during the attacks was stertorous, and following the attacks it was labored. The pulse was fast and of good quality. The patient made signs to tell that she had a visual aura and a choking sensation before the onset of the convulsions.

From the patient's sister, the following history was obtained. The patient's birth was spontaneous and rapid. In the course of the excitement that followed the birth, the baby was dropped to the floor from a height of about 30 inches (80 cm.). It fell on its head and remained unconscious for several hours, so that those present thought that it was dead. Immediately after regaining consciousness, it began to have convulsions. The godmother, who was present at the delivery, stated that at birth the baby was normal.

The child developed normally, except for frequent attacks of headache followed by fainting spells. These attacks persisted after the patient became mature. They were of short duration and occurred at intervals varying from one to three months. After her marriage, fourteen months previous to her admission to the hospital, the attacks became more and more severe, completely exhausting the patient, and she died from bronchopneumonia.

Autopsy.—On external examination, the head showed nothing abnormal. There were several scars on the lateral margin of the tongue. The skull was symmetrical, and the dura mater was smooth and tightly stretched over the posterior half of the left hemisphere.

The brain weighed 1,305 Gm. The left occipital lobe was the site of a cyst which occupied the convex surface, extending posteriorly from a line connecting the anterior occipital sulcus with the parieto-occipital fissure to the pole of the lobe. On the median and basal aspect, the occipital convolutions were preserved, but appeared flattened. The cyst was covered by a thin, transparent membrane, which seemed to be the direct continuation of the leptomeningi. The sagittal diameter of the cyst was 5 cm., the transverse diameter 4 cm. and the vertical diameter 3.5 cm. The content was a clear, colorless fluid, which escaped when the medulla oblongata was severed so that the cyst collapsed.

A sagittal section through the middle of the left hemisphere (fig. 1) revealed that the cyst had resulted from a funnel-shaped defect in the brain substance, which communicated with the posterior horn of the left lateral ventricle. The narrow end of the funnel measured 10 mm. in diameter and opened into the ventricle. The membrane on the surface contained several irregular, moderately firm, pale yellowish-gray areas, from 3 to 6 mm. in diameter and from 0.5 to 1 mm. in thickness. At the edge of the defect, the membrane passed into the leptomeningi, but a thin layer of grayish-white brain tissue was attached to its internal surface as it became free, and gradually merged with it.

The lateral wall of the defect was formed by smooth white brain substance. A delicate network of branched blood vessels extended from the lateral wall through the cavity to the surface membrane. The ependyma of the ventricles was smooth, and there was a marked dilatation of the posterior horn of the left lateral ventricle.

Coronal sections through the other parts of the brain did not reveal anything abnormal.

The rest of the observations made at autopsy were as follows: confluent bronchopneumonia of both lower pulmonary lobes; cloudy swelling of the liver, with areas of fatty degeneration; acute tumor of the spleen; solitary cyst, 4 by 5 by 2.5 cm. in diameter, in the upper pole of the left kidney; menstruating uterus; small follicular cysts in both ovaries; and a hemorrhagic corpus luteum in the right ovary.

Microscopic Observations in the Brain.—From the white matter of the occipital lobe, the cyst was separated by a membrane of dense fibrillar glia. The membrane was about 0.1 mm. thick and did not contain any nerve fibers. It was covered

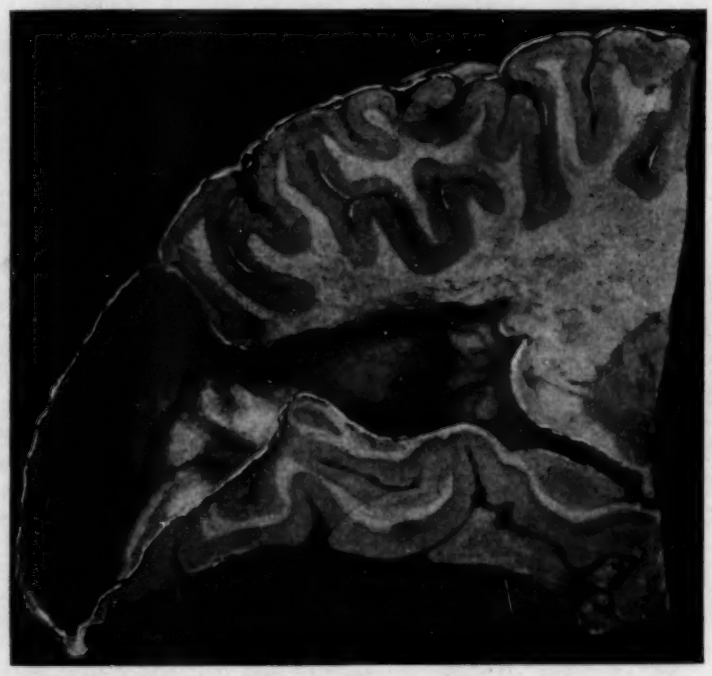

Fig. 1.—A porencephalic cyst in the left occipital lobe communicating with the posterior horn of the lateral ventricle. The drawing is after the specimen, which was preserved in Kaiserling solution.

on the inside by a very low cuboidal epithelium. The parietal gliosis continued into the lateral ventricle. The convolutions that took part in walling off the cyst were narrow and tapered into the membrane that formed the roof of the cyst (fig. 2). Nowhere did the gray matter border on the cyst, but the latter was covered on the inside by a thin layer of medullary nerve fibers and glia. The cyto-architecture of the cortex was preserved only in the part adjacent to the valley of the convolution, while toward the surface the ganglion cells became more and more scanty and lost their arrangement to parallel horizontal layers. Some of the ganglion cells were shrunken and surrounded by neuronophages. The tapering part of the convolution was completely devoid of ganglion cells, their place being taken by fibrillar glia and medullary nerve fibers, which ran in

various directions and showed varicose swellings. Where the cortex passed into the surface membrane, the cyst possessed a distinct cuboidal epithelium (fig. 2 *E*).

The free membrane on the surface of the cyst had an average thickness of 220 microns. It consisted of two layers, an outer one of connective tissue with blood vessels and single mononuclear cells and an inner one made up of fibrillar glia. The inner layer varied in thickness and in places swelled up to form plaques, which were as much as 800 microns thick. The glia contained only a few nuclei and capillary blood vessels. The plaques were more cellular, the cells accumulating in the outer third. They had round nuclei with a finely granular chromatin net and centrally located nucleoli, which took the basic stain. Many of these cells

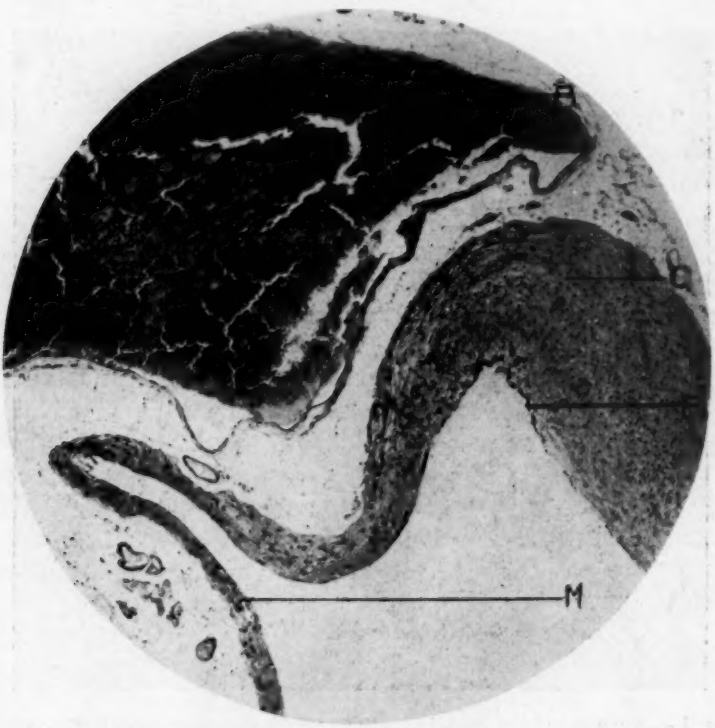

Fig. 2.—A photomicrograph illustrating the relations of the glial part of the porencephalic membrane to the lateral wall of the cyst. Note the tapering of the cortex (*C*) into the membrane (*M*) and the distinct epithelial lining (*E*). The dark area in the upper half of the field (*B*) is a dilated meningeal vein. The material was fixed with a diluted solution of formaldehyde, U. S. P. (1:10). The section was stained with hemalum-eosin; magnification, 60 times.

were filled with dark brown and yellowish-brown pigment granules (fig. 3). With Turnbull's reaction for iron, the dark brown granules stained deep blue, while the yellowish-brown granules either remained unstained or appeared a light green. Outside the plaques there was only a little pigment. Nerve cells could not be detected nor did specific stains (Bielschowsky, Alzheimer, Weigert-Pal, Kultzinsky, Spielmeier) demonstrate any nerve fibers. Here and there one found small spherical bodies that gave the microchemical reaction of calcium.

Bundles of glia fibrils, from 40 to 150 microns thick, extended from the glial part of the membrane into the outer zone of connective tissue. In transverse sections, these bundles appeared as whorls surrounded by a capsule of connective tissue. This ingrowth of glia into the mesenchyma was most marked at the edges of the cyst where the bundles reached a length of 2.5 mm., crossed the sulcus and extended to the vertex of the next convolution.

The mesenchyma part of the membrane continued as pia and arachnoidea over the adjacent convolutions. Where it filled the sulcus that separated the cyst from the rest of the brain, it contained accumulations of large oval cells, many of which had engulfed dark brown pigment granules.

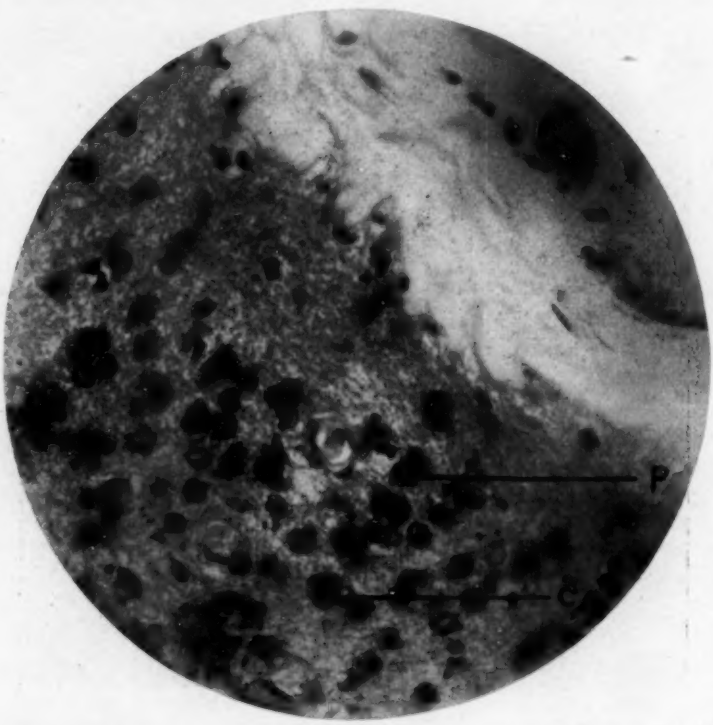

Fig. 3.—Deposits of blood pigment (*P*) in the large glia cells (*C*) of a plaque-like thickening of the membrane. The technic is outlined in the legend for figure 2; magnification, 450 times.

In the convolutions adjacent to the defect, which on gross examination appeared normal, cone-shaped, sharply circumscribed areas of gliosis were found (fig. 4). They were located in the vertex of the convolutions and extended through the entire thickness of the ganglion cell layers, the base of the cone fusing with the molecular layer, and the apex extending into the medulla. The areas measured 3 mm. in length and 1 mm. in depth, and, in addition to fibrillar glia, contained loose bundles of medullary nerve fibers, which crossed each other in various directions and were connected with the fibers of the central part of the convolution. There were no nerve cells in these areas.

Sections were taken from the central and paracentral convolutions, the temporal lobes, the insula Reili, the corpus callosum, the choroid plexus, the pons, the corpora quadrigemina, the pedunculi cerebri, the cerebellum, the nucleus caudatus, the thalamus opticus, the putamen, the globus pallidus and the nucleus lentiformis. They did not reveal any abnormalities, except a moderate thickening of the leptomeninges over the cerebral hemispheres.

COMMENT

The trauma from which the patient had suffered immediately after she was born seemed to be the most reasonable explanation for the

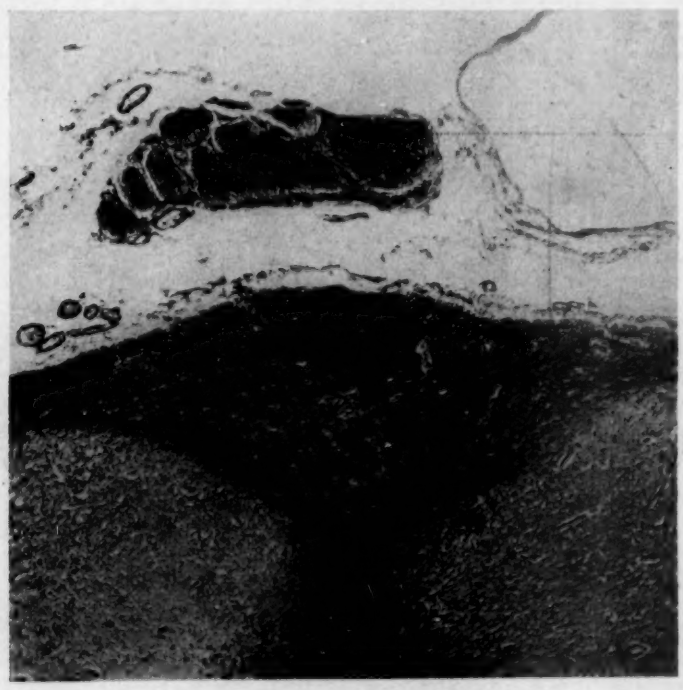

Fig. 4.—A cone-shaped area of gliosis in the cortex of a convolution adjacent to the defect. Above the area is a transverse section through a bundle of glia tissue distinctly separated from the cortex of the brain. In serial sections, this bundle could be traced to the glial part of the porencephalic membrane. The material was fixed with a diluted solution of formaldehyde, U. S. P. (1:10). Mallory's stain (phosphotungstic hematoxylin) was used; magnification, 60 times.

large cyst of the occipital lobe. The birth was spontaneous and rapid, and though precipitate birth does not seldom lead to injuries of the brain, the close association of the first clinical manifestations with the accident speaks strongly in favor of the postnatal origin of the initial lesion. The cyst showed the macroscopic and microscopic picture that is typical of porencephaly. The most common location of porencephaly

is the central convolutions and the insula of Reil, the parietal region being especially exposed to birth injuries. In about 14 per cent of the cases, the occipital lobe is found affected (Siegmund). Porencephaly from birth trauma is frequently bilateral and is often combined with other structural changes of the brain, such as microgyria, nodular thickenings of the ependyma and glial scars in the cerebral cortex and central ganglia. In the present case, the defect was unilateral.

The literature on porencephaly contains the records of a number of cases in which the defect could be traced to a trauma in the postnatal life. The age at which the trauma had occurred varied greatly. Thus Ponfick [13] reported a case of porencephaly in a man aged 23, who at the age of 3 years had been hit on the head by the vanes of a windmill. Kopp's [10] patient, who was successfully operated on for a large porencephalic cyst when he was 16, had fallen from the third floor when he was 3. Struchlik-Sirotow's [15] patient, a man aged 37, who had a large cyst in the region of the left central convolutions, had been run over by a car at the age of 7. In Boettger's [7] case, the accident had occurred when the patient was 17 (with death at 66); in Schroer's [14] case, at the age of 48 with death at 58, and in von Kahlden's,[4] at the age of 45 (with death at 50).

The question will arise whether there are differences in the reactive changes of the central nervous tissue, as evidenced by the structure of the wall of the cyst, between the defects which form in the immature brain and those which develop after the organ has attained its morphologic maturity. The studies of Spatz [31] seem to suggest such differences. Spatz compared the effect of sewering the spinal cord in new-born with that in adult rabbits, and found that in the immature central nervous tissue there was a rapid liquefaction of the traumatized area and a quick removal of the debris, while the reactive changes of the glia and especially of the mesenchyma were insignificant. In the adult, glial and mesenchyma proliferation predominated over the liquefaction and led to scars composed of glia and connective tissue. Cysts formed in the mature central nervous system had a distinct wall that gradually passed into the adjacent normal tissue, whereas porencephalic cysts originating in fetal life, during birth or in early infancy had a thin wall that was sharply separated from the normal brain. The immature brain, he thought, possesses a great adaptability, which permits destructions so extensive that if they occurred in the brain after it reached maturity they would be incompatible with life. He concluded that it is the differentiation of the medullary tissue that determines the form of reaction.

Spatz's conclusions were criticized by Schwartz. Schwartz, to whom great credit is due for calling attention to the tremendous

31. Spatz, H.: Ueber eine besondere Reaktionsweise des unreifen Zentralnervengewebes, Ztschr. f. d. ges. Neurol. u. Psychiat. **53**:363, 1920.

importance of injuries of the brain at birth, pointed out that Spatz performed his experiments on new-born rabbits, in which the central nervous system is much less mature than even in the youngest premature infant. Many injuries of the immature brain do not lead to cysts, but to glial scars. If the large porencephalic defects show a lack of reactive changes, it is due to their bordering on regions of the brain with different functional properties and a different vascular supply. In destructive processes of the adult brain a similar lack of reaction is observed, if they involve a functional and structural unit of the brain, for instance, the centrum semiovale. In typical porencephaly, however, as in the case reported, the fact that the mesenchyma does not take part in the walling off of the defect remains an outstanding feature and it has yet to be proved that the same indifference of the connective tissue to acute lesions of the brain may also occur in the mature organ.

Communication with the ventricles is found in both forms of porencephaly, in the early ones as well as in those acquired in later life. The initial lesion may extend into the ventricle, or a cyst at first separated from it by a thin membrane may later break into the ventricle by gradual growth from an increasing accumulation of fluid, since the thin wall permits expansion.

Three histologic observations may be discussed in detail; namely, the structure of the membrane that covers the defect, the deposits of blood pigment in this membrane and the adjacent leptomeningi, and the cortical areas composed of glia and medullary nerve fibers in the convolutions near the cyst.

Most of the investigators who have studied microscopically the porencephalic membrane have emphasized that a thin layer of nervous tissue was attached to the inside of the thickened leptomeningi. Siegmund believed that the porencephalic cyst never borders directly on mesenchyma tissue, but that it is always separated from it by a layer of brain tissue. Langhans,[32] who studied the membrane of a cyst operated on by Kocher, found on the surface sclerosed and vascular connective tissue while the rest was made up of a loose fibrillar tissue resembling glia. In the case observed by Frensdorf,[33] the membrane contained glia and medullary fibers. Bornhaupt [34] and Delore and Pic observed, in the glia, cells that looked like nerve cells. Seitz spoke of a layer of brain tissue of varying thickness, and Kopp described connective tissue and glia. In my case, the porencephalic membrane

32. Langhans, quoted from Kopp (footnote 10).

33. Frensdorf: Fall von Porencephalie und Ulegyrie (Narbenbildung in der Rinde) auf vaskulärer Basis, Verhandl. d. Irrenärzte Niedersachsens und Westphalens, Session of May 5, 1923.

34. Bornhaupt, L.: Hirncyste des rechten Seitenventrikels operativ geheilt, Zentralbl. f. Chir. **45**:404, 1918.

contained only glia. Nerve cells and nerve fibers could not be demonstrated.

It seems that the glial part of the membrane is the remnant of the plexiform layer of the cortex, the other layers of the cortex and the medullary tissue of the convolutions and of the centrum semiovale being replaced by the cyst. According to Schwartz, the different layers of the cortex possess a certain independence from each other, which explains why the plexiform layer may remain when the other parts of the cortex are destroyed by hemorrhage or softening.

There exists another lesion of the brain resulting from injuries at birth, which, too, is often associated with large cysts on the surface or the base of the hemispheres, namely, lobar atrophy (lobar sclerosis). The pathogenesis of these cysts, however, is different from that of the porencephalic cysts, from which they should be distinguished. While in porencephaly it is a softening and liquefaction of the entire brain substance that leads to the formation of the cyst, in lobar atrophy the initial lesion is a loosening of the cortical and medullary tissue with a more or less complete destruction of the nerve fibers and ganglion cells followed by proliferation of the glia, sclerosis and shrinking (Schwartz). The shrinking of circumscribed, often large, areas of the brain produces depressions on the surface of the hemispheres, which are filled by cysts. The cysts contain a clear fluid and, on superficial examination, resemble porencephalic cysts. They are located, however, between the leptomeninges, the arachnoid membrane bridging the defect, while the pia mater is attached to the shrunken convolutions. In the external membrane of these cysts, I have not found any glia tissue, which I think is an important difference between the porencephalic cysts and the cysts over the atrophic parts of the brain in lobar atrophy.

In the porencephalic membrane, I have described bundles of glia extending from the ectodermic part into the leptomeningi. This ingrowth of glia into mesenchyma has been repeatedly observed. Globus saw it in a case of porencephaly with encephalitic changes. In a baby, aged 15 months, with porencephaly and micro-encephaly, Babonneix and Lhermitte [23] found dense plaques rich in medullary nerve fibers, which were located in the thickened pia mater and were distinctly separated from the cortex of the brain. In the case under discussion, the bundles of glia in the leptomeningi did not contain any nerve fibers.

Many of the reports on porencephaly mention a brownish pigmentation in and about the defect. This pigmentation was observed by the early investigators (Heschl, Rogers,[35] Birch-Hirschfeld,[36] Chiari [37]

35. Roger: Ueber Porencephalie, Inaugural Dissertation, Erlangen, 1866.

36. Birch-Hirschfeld, F. V.: Ueber einen Fall von Hirndefekt infolge von Hydrops septi pellucidi, Arch. f. Heilk. **6**:1, 1867.

37. Chiari, H.: Aus der Prosektur des St. Anna Kinderspitals in Wien, Jahrb. f. Kinderh. **15**:330, 1880.

and others) and was sometimes considered proof of the traumatic origin of the defect. In my case, there were deposits of iron-containing and iron-free pigment in the plaques of the membrane and in the meningi adjacent to the cyst. Is this pigment a remnant of the hemorrhages caused by the trauma inmmediately after birth? It is hardly possible that blood pigment would be stored over so long a period of time, and another explanation seems to be more likely. In the introduction, I mentioned that the lesions of the brain from birth injuries remain points of diminished resistance in which later circulatory disturbances are apt to occur. Such circulatory disturbances may lead to hemorrhages months or even years after the injury. It is undoubtedly from these late hemorrhages that the pigmentation of the porencephalic cyst is derived.

Relatively recent small hemorrhages into the leptomeningi also account for the cellular accumulations in the meningi near the cyst. It is well known that extravasations of blood into the meningi cause a proliferation of the local histiocytes, which engulf the free red cells and their debris, transforming them into pigment.

Recently I studied the brain of a premature child, 7 months of age, who since birth had been suffering from a stiffness of the neck and of the back. This stiffness diminished, later to return with increased intensity. At necropsy, recent and old hemorrhages were found about the frontal lobes, the convolutions of which were narrow and separated by deep sulci. There was an extensive calcification of both suprarenal cortices. The microscopic examination of the brain revealed circumscribed areas of gliosis in the cortex of the frontal convolutions and in the upper part of the thalami optici. In the latter location there were numerous calcified ganglion cells. About the scars in the thalami optici there were recent hemorrhages surrounding dilated capillaries and venules.

The cortical areas of gliosis were of the same microscopic structure as were those in the case of porencephaly. There was a dense network of fibrillar glia containing thin medullary nerve fibers that were connected with the medulla of the convolutions. These areas resembled the "plaques fibro-myeliniques" described by Vogt and Vogt,[38] who found them frequently in the cortex of normal brains, in which they were so small and scanty as to cause no functional disturbances, being a mere incidental observation. In his studies on the myelinization of the cerebral cortex, Goichi [39] observed in the frontal gyri of two infants, aged 6 and 8 months, circumscribed areas of premature myelinization.

38. Vogt, O., and Vogt, C.: Zur Lehre der Erkrankungen des striären Systems, J. f. Psychol. u. Neurol. **25**:633, 1920.

39. Goichi, Hirako: Ueber Myelinisation in der Grosshirnrinde, Schweiz. Arch. f. Neurol. u. Psychiat. **9**:275, 1921.

Goichi explained these foci on the basis of a locally increased blood supply, following von Monakow's conception of the correlation between vascularization and the development of the medullary sheaths.

In their case of porencephaly referred to in a preceding chapter, Babonneix and Lhermitte emphasized the presence in the cerebral cortex of well developed plaques of glia and medullary fibers. This observation and the cases that I have discussed seem to support an explanation first given by Schwartz, namely, that the "plaques fibro-myeliniques" may result from circumscribed areas of loosening of the cortical tissue caused by injuries of the immature organ. It is possible that the increased vascularization during the organization that follows the loosening of the brain substance accounts for the local overgrowth of medullary fibers. An excessive ingrowth of medullary fibers sometimes is also observed in the central ganglia, especially in the corpus striatum, giving rise to a peculiar change in the structure of the gray matter, the so-called "état marbre" (status marmoratus of Vogt and Vogt).

SUMMARY

A case of porencephaly in a woman, aged 22, is reported, which illustrates the great importance of the trauma in the etiology of this rare and interesting lesion. The trauma occurred immediately after birth and in its action on the brain could therefore be compared with the injuries that occur during birth. The histologic observations are discussed, especially in connection with the question of the reparatory processes in the immature central nervous tissue.

EXTRADURAL LIPOMA OF THE SPINAL CANAL*

J. A. KASPER, M.D.
AND
A. COWAN, M.D.
DETROIT

Extradural spinal lipoma is comparatively uncommon. Stookey,[1] in reviewing the literature to 1926, was able to find reports of only nine cases. He referred to a report by Chapelle [2] as being the first description of this type of tumor. In a recent report on a series of 179 extradural spinal tumors, Elsberg [3] recorded only one example of lipoma.

Being a benign tumor of soft consistence, lipoma presents a difficulty in roentgenologic diagnosis. This probably accounts for its infrequent recognition. It may be present without symptoms, unless it grows to a great size; then it produces pressure on the cord (Chapelle,[2] Obré,[4] Elsberg [3]).

For these reasons, and particularly because of an unusual clinical course, the present case is considered of sufficient interest to be reported.

REPORT OF CASE

History.—R. D., a white boy, aged 6, was brought to this hospital to the pavilion for patients with meningitis during the recent epidemic of meningococcus meningitis in this region. His family physician had made a diagnosis of "suspected meningitis." At the time of admission, the temperature was 102.4 F. (rectal); the pulse rate was 120, and the respiration rate 32 per minute. He was irritable and irrational.

The onset of the present illness occurred four days before his admission to the hospital. The illness began with fever and sore throat. Two days after the onset, the patient vomited and complained of headache.

The past history was negative. Previous to the present illness the boy was in good health.

Physical Examination.—The positive observations were redness of the throat, an indefinite Babinski sign and exaggerated knee reflexes. No abnormal signs

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* From the Pathological Laboratory and the Division of Communicable Diseases, Herman Kiefer Hospital, Department of Health.

1. Stookey, B.: Intradural Spinal Lipoma, Arch. Neurol. & Psychiat. **18**:16 (July) 1927.

2. Chapelle: Extradurales Lipôme, Bull. Soc. anat. **22**:6, 1847; quoted by Stookey.

3. Elsberg, C. A.: Extradural Spinal Tumors, Surg. Gynec. Obst. **46**:1, 1928.

4. Obré: Lipoma of the Meninges, Tr. Path. Soc. London **3**:248, 1852; quoted by Stookey.

were discovered in the chest. A spinal puncture was done, and 10 cc. of clear fluid was obtained. This was under slightly increased pressure and was followed by blood.

Examination of the spinal fluid showed a slight globulin reaction. Fehling's reaction was positive. No bacteria were found on direct smear or culture.

The observations did not warrant a positive diagnosis of meningitis, but the child was considered to be in a critical condition. He continued to be irrational and six hours after admission became cyanotic, and the respirations were labored. He became progressively weaker, and expired thirteen hours after admission.

Autopsy.—Autopsy was performed seven hours after death. Only the important observations are recorded.

The pupils were equal and regular, measuring 4 mm. in diameter. The sclerae were clear. In cutting through the skull below the occipitoparietal suture, the dura was cut, and immediately dark fluid blood began to flow out. This was collected in a basin, and the amount was estimated to be between 200 and 250 cc. When the calvarium was removed, the source of this blood was found to be in the spinal canal. The meningeal vessels were filled with blood. These were intact, as were the sinuses. There was no fracture in the skull. The pia and the arachnoid were transparent over the entire brain. No exudate was apparent. The brain was deep pink, moderately dry and firm. On section, the cortex of the cerebrum was found to be somewhat swollen. The cerebral capillaries were rather prominent. Perivascular extravasation of blood could not be found about any of them. The ventricles contained clear fluid. The spinal cord was exposed by removing the vertebral laminae. On the posterior surface of the dura were two masses of firmly adherent, dark pink, soft, friable substance which could not be removed en masse. The upper of these masses was in the region between the second cervical and the eighth dorsal vertebra. The lower was between the third lumbar and the second sacral vertebra. At the level of the fourth lumbar vertebra was clotted blood. Extravasated blood was also found within and around the extradural tumor. Where the needle for spinal puncture had entered through the dura, clotted blood was found on the inner surface. Reflection of the dura showed the cord to be slightly blood tinged. The tumor mass apparently had filled the space between the dura and the posterior wall of the spinal canal because the removed laminae contained it. It was firmly attached in the depressions between them. The other observation of importance was that of gray hepatization of the upper lobe of the right lung.

The anatomic diagnosis was pneumonia of the right upper lobe; hemorrhage into the spinal canal, and extradural glioma or lipoma.

Microscopic Examination.—Spinal Tumor: Several sections from different portions revealed fat tissue with moderately large vessels, which were filled with blood. There was rather extensive extravasation of blood into the mass of fat. A large blood clot was present on the external surface of the mass in one section. A section containing dura showed the fat tissue to be in close approximation on one surface. The opposite surface was smooth.

Cerebrum: Moderate edema was present throughout. The peripheral portion of the cortex showed slight necrosis. The pia was normal. No exudate was found in the arachnoid space. There was diffuse cerebral capillary dilatation and engorgement.

Right Lung: All alveolar capillaries were prominent and engorged. Most of the alveoli were filled with leukocytes, chiefly polymorphonuclears. All other alveoli contained thin fluid with few leukocytes and some fibrin.

The histologic diagnosis was extradural lipoma with hemorrhage; acute congestion of the cerebrum, and lobar pneumonia of the right lung.

Roentgen Examination.—The removed laminae containing the tumor were shown by x-ray to be normal. The study was made in two planes.

COMMENT

In reviewing the clinical course in this case, an explanation of the symptoms can be ventured on the basis of the anatomic observations. The irritability and the irrational state of the patient can be explained as having been due to a cerebral irritation of the nature of a meningism, caused by the toxemia resulting from the infection in the right lung.

The terminal cyanosis and respiratory distress were in all probability due to the intracranial pressure which was caused by the hemorrhage into the spinal canal following the spinal puncture.

Lipoma in the spinal canal often has its origin in the fat which is present about the vertebrae and even if this is located external to the canal,[5] penetration between the vertebrae may take place. The case which Elsberg [3] reported was of this type, since it was associated with multiple lipomatosis.

The chief interest evoked by the clinical course in this case is in the fact that a lipoma, which under ordinary conditions would be of little importance, has to be recognized as an indirect cause of sudden death following the employment of a valuable aid in diagnosis, namely, spinal puncture.

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5. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1919, p. 424.

THE ISSUES AT STAKE IN THE GRADING OF TUMORS*

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PHILADELPHIA

To me, as to most pathologists, has often come the desire to classify the material coming to my hands, as much for my own benefit as for that of others similarly interested. This has been particularly true of tumors.

The recent developments and revivals in the literature of the systems of so-called "grading" of carcinomas naturally intensified the original urge. I began to classify the 890 carcinomas of the breast available to me, and soon reached the appalling number of 500 different types; it became evident that such refinements (?) of analysis would lead to the absurd (?) result of 890 types of carcinoma in 890 breasts.

This study, together with that of tumors of other organs, again brought out the well known fact that there is as yet no adequate definition of a tumor. The only one which begins to satisfy is the total contents of a good monograph on malignant growths. Even this is inadequate, for until the biology of tumors is known, no definition can be inclusive.

This situation tends to restrict one to classifying tumors solely as "benign" or "malignant," and with knowledge of the later history of patients, obtained from a follow-up service, one must mentally hedge in this in some cases.

In view of the prominence occupied by tumor grading and my own experience with it, it seemed that the time had come for another examination into its rationale.

I have chosen to do this by first presenting the facts emerging from an investigation of the material collected over many years here at the Lankenau Hospital, and then discussing their significance in terms of tumor grading.

One hundred consecutive cases of carcinoma of the breast, dating from September, 1920, were taken for study. One hundred and five were necessary to complete the list, for in five cases the follow-up records were incomplete. It is clear that in order to judge of the validity of tumor grading the later history of the patient from whom the specimen was taken is essential.

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* Submitted for publication, June 10, 1929.

* From the Research Institute of the Lankenau Hospital.

* This paper and the articles by Hammett and Ingleby, which were published in the October issue, and complete references to which will be found in this article, are supplementary and complementary to each other.

THE MANY VARIABLES THAT HELP TO DETERMINE THE HISTOLOGIC CHARACTER OF CARCINOMA

The first matter to be considered is the histologic character of the tissue removed. The ideas in regard to this almost choke the literature, which has recently been well reviewed by Plaut.[1]

In table 1, I have given the histologic pictures presented by each of my 100 specimens. From these, it is evident that a great diversity is exhibited. Every experienced pathologist knows that what is true of carcinoma of the breast is true of carcinoma of other organs, the differences being equally impressive when details are sought. So results

TABLE 1.—*The Histologic Pictures of Tumor as Determined in Specimens of Carcinoma of the Breast from 100 Patients*

	Dead of Metastasis (61)		Dead of Other Causes (18)		Living with Recurrence (4)		Living and Well (17)	
	Percentage	Actual Figures	Percentage	Actual Figures	Percentage	Actual Figures	Percentage	Actual Figures
Small cells	29	18	33	6	..	0	23	4
Large cells	81	50	77	14	75	3	47	8
Necrosis: Sparse	29	18	5	1	50	2	6	1
Massive	14	9	33	6	..	0	..	0
Small nests: Loose	24	15	5	1	..	0	17	3
Confined	24	15	39	7	25	1	17	3
Large nests: Loose	18	11	11	2	25	1	6	1
Confined	39	24	39	7	..	0	17	3
Angular nests	11	7	5	1	..	0	17	3
Rounded nests	2	1	5	1	..	0	..	0
Thin streaks	47	29	27	5	75	3	53	9
Thick streaks	36	22	22	4	25	1	35	6
Stroma:								
Fibrous, cells; Sparse	19	12	16	3	25	1	6	1
Profuse	13	8	5	1	..	0	..	0
Acute	28	17	16	3	25	1	6	1
Chronic	24	15	11	2	25	1	11	2
Thrombosis: Lymph vessels	34	21	22	4	50	2	17	3
Blood vessels	6	4	..	0	25	1	..	0
Metastasis: Alike	34	21	44	8	50	2	11	2
Denser	24	15	11	2	..	0	17	3
Looser	..	0	..	0	..	0	..	0
Unlike	5	3	..	0	..	0	..	0

from the analysis of these carcinomas of the breast are applicable to tumors in general.

What is it that determines the differences?

Cell Size.—The cell size is dependent on at least four factors: (1) the kind of cells, i. e., epithelial and their derivatives, etc.; (2) the structural environment, i. e., compression by dense tissue or freedom for expansion in loose; (3) the chemical environment, i. e., nutrition, toxic products, etc., and (4) the reproductive activity. Hammett[2] showed that cell size is inversely proportional to the rate of cell pro-

1. Plaut, A.: The Relation Between the Histologic Picture and the Prognosis of Tumors, Arch. Path. **3**:240 (Feb.) 1927.

2. Hammett, F. S.: Cell Division and Cell Growth in Size, Protoplasma, to be published.

liferation. The coefficient of correlation in the meristem of root tips of *Zea mays* is —0.577 —0.075.

On the biologic basis, then, small cells should be and are considered more "malignant" than large. But here the difficulty enters that small cells may result from lack of room to grow, or compression, and might not, indeed, represent a high rate of proliferation.

The omission of the state of the nucleus from this analysis is purposeful. The usual methods of taking the specimen and the way it is handled prior to fixation preclude the obtaining of any valid index of nuclear activity as of the time at removal. But even if one could obtain ideally fixed and stained preparations, who has time or opportunity to study serial sections of the tumors engulfing one from a busy surgical clinic? Besides, they would be of only one instant in the life of the carcinoma, i. e., when it was removed.

Architecture.—The architecture of the growth is dependent on at least eleven factors. The first four are the same as those influencing cell size. In addition, there are: (5) the adhesive property of the cells, which determines whether they stick together in larger or smaller nests, sheets or streaks; (6) the "erosive" power of the growth, as when a tumor eats away bone; (7) the "invasive" power of the cells as they insinuate themselves throughout the contiguous tissue of the host—this may be counteracted by compression exerted by the surrounding tissues or by their relative impenetrability; (8) the vascularity, i. e., the abundance or sparseness of blood and lymph channels and their size—included in this might be the relative amount of duct spaces; (9) organization factors, i. e., those which cause the growths to appear in discrete forms like ducts, acini, etc., as in adenocarcinoma; (10) secondary changes of all types, i. e., degeneration, necrosis, hemorrhage, hyalinization, calcification, pseudocysts and the formation of specific by-products of cellular activity, such as mucus; and (11) the architecture and the secondary changes in the surrounding tissue.

Stroma Reaction.—The reaction of the stroma depends on at least three factors: (1) the organ or the tissue in which the tumor is growing; (2) the physiologic state of the organ or the tissue in which the tumor is growing, and (3) the secondary changes in the tumor itself.

I found, as did Greenough [3] and others,[4] that cellular infiltration is particularly correlated with the degree of necrosis and degeneration in the tumor, and not with the hypothetic "resistance" to the growth as determined by the end-results.

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3. Greenough, R. B.: Varying Degrees of Malignancy in Cancer of the Breast, J. Cancer Research **9**:453, 1925.

4. Hueper, W. C.: Carcinomas of the Uterine Cervix, Arch. Path. **6**:1064 (Dec.) 1928.

One should dispose of the idea that the stroma reaction is due to the cancer cell as a "foreign body," for the reaction is not specific to degenerating or necrotic cancer cells, but is found wherever disintegration of tissue takes place, and is not found in cancer save when necrosis is in progress. On the biologic basis the concept is unsound also. For if those who have made a study of tumors are correct in their belief that the cells of most carcinomas are derivatives of preexisting normal cells, the most obvious point of difference is lack of restriction to multiplication and cancer cells are, therefore, not "foreign bodies." For to consider the cancer cell as a "foreign body" would be to believe that it is a biologic "sport" or mutation. No evidence for this is available. That the carcinoma cell undergoes degeneration and then becomes toxic as does any other cell under like conditions is, therefore, more readily explained on environmental than on hereditary grounds, at least at present. Certainly, if it were a foreign body, immunologic reactions should be demonstrable, but thus far all attempts in this direction have been unsuccessful. Nevertheless, the interesting results of Gruskin [5] may be consulted, in which the embryonic cell concept is supported, and the "immunologic" work of Lumsden [6] on sarcoma in rats; sarcoma in rats is, however, far different from carcinoma in man.

Finally, as far as this phase is concerned, I cannot bring myself to the belief that carcinomas obey their own rules and have nothing to do with the laws that govern normal growth. All my sympathies are with attempts to discover the laws of normal growth in the belief that abnormal growths behave differently only in detail. This subject is discussed at length by Hammett.[7]

Considering the breast as a physiologic structure, I find the following factors to be of prime importance: Signs of functional strivings are present even at birth. From the onset of puberty until the menopause (and after) the breasts of women are never physiologically quiescent. Nearly every woman at practically every menstrual cycle is aware of this fact. The most potent stimulus to activity of the breast is, of course, pregnancy. The anatomic reactions are hypertrophy and hyperplasia of the gland tissue and regression of the connective tissue. There is ever-increasing budding of the terminal ends of the ducts and

5. Gruskin, B.: A Serum Test for the Diagnosis of Cancer Based on a New Theory of Etiology, Am. J. M. Sc. **177**:479, 1929.

6. Lumsden, T.: Chemotherapy by Means of Vaccine Treatment and Immunity, Rep. Internat. Conference on Cancer, London, 1928, p. 216.

7. Hammett, F. S.: An Interpretation of Malignant Growth Based on the Chemistry of Cell Division, Arch. Path. **8**:575, 1929. For a discussion from the morphologic point of view, see Müller, Heinrich: Eine einheitliche Erklarung fur die im menschlichen Korper vorkommenden geweblichen Neubildungen, Virchows Arch. f. path. Anat. **269**:105, 1928; Die histologische Ubereinstimmung zwischen Epithelregeneration und Krebsbildung, Ztschr. f. Krebsforsch. **28**:383, 1929.

a coincident disappearance of connective tissue, probably to make room. When lactation is interrupted the reverse occurs: the connective tissue undergoes hyperplasia and the glandular tissue disappears, until finally the perilobular connective tissue again surrounds small lobules at the ends of the ducts.

It is now fairly well established that the menstrual changes are the same as those of pregnancy, only less in degree. This means that in the normal breast there is a constant reciprocal growth and recession of epithelium and connective tissue.[8]

These normal physiologic changes of menstruation cannot help but be important factors in the determination of the histologic picture in mammary carcinoma. They also must be significant factors in determining the type of growth that occurs.

Considerable degrees of fibroblast reaction may, indeed, not be, as is often stated, a "defense" reaction on the part of the adjacent tissues, but can be the normal menstrual hyperplasias. Also, in the normal breast, small "round" cells are in the picture of every lobule. Most of these are not inflammatory in origin, but are epithelial cells in some stage of normal progression or regression. They look like inflammatory cells at first glance, and hence may be misinterpreted as a "defense" reaction.

Furthermore, it is well known biologically that cell reproduction can take place at almost any stage of the life of the cell. That is, the two cells that result from division of a mother cell can immediately undergo division without waiting to grow. Further, old and adult cells are still capable of dividing to repair wounds under the proper stimulus. Hence, this property may well be a factor in the production of the diversity of anatomic types of carcinoma exhibited in this or any other similar material.

Thus, when certain epithelial cells become carcinomatous, they may conceivably begin to divide at any stage they happen to be in. If the parent cells of a carcinoma are full grown and have reached the stage of forming an acinus, or duct, the daughter cells may also be large and form an adenocarcinoma. If, on the other hand, the mother cell gives rise to daughter cells before full differentiation, the carcinoma will be composed of smaller cells not arranged in architecturally definite form.

The pathologic fact that adenocarcinomas of the breast occur less frequently than other types, may, on grounds of cell heredity, be due to the fact that the mature stage of the cells in the breast lobule is of shorter duration than the preceding and succeeding phases of cell differ-

8. McFarland, J.: Residual Lactation Acini in the Female Breast, Arch. Surg. **5**:1 (July) 1922. Reimann, Stanley P.: Kaufmann's Pathology [tr.], Philadelphia, P. Blakiston's Son & Company, 1929, p. 1766.

entiation. Besides, the stimulus to grow is closer to immature cells just divided (as in menstrual hyperplasia) than when they are fully grown.

Furthermore, the gross spread of a carcinoma of the breast is undoubtedly influenced by the physiologic state of the organ. In the first place, the breast is periodically stimulated to grow, and it is possible that this normal stimulus to cell proliferation exerts an additional effect on the division of the carcinoma cells. On the other hand, the inhibitors of cell reproduction, which normally restrict untoward menstrual hyperplasia, may retard the proliferation of the carcinomatous cells. Thus, the growth picture would vary according to the respective stimuli arising from the normal physiologic processes.

Then during the epithelial hyperplastic stage, the ducts tend to be more dilated and to increase in number. This affords increased opportunity for a spread of the malignant growth in these channels. The disappearance of some fibrous tissue at this stage and the general loosening of the tissues yields a similar opportunity for spread in other channels. During the fibrous hyperplastic stage, when the ducts tend to become constricted, and the tissues tighten up again, hindrance to spread is increased. The coincident changes in vascularity obviously provide at times a more, and at times a less, plentiful supply of materials for growth.[9]

OUTCOME OF ATTEMPTS TO GRADE ONE HUNDRED CARCINOMAS ON HISTOLOGIC OBSERVATIONS ALONE

In the foregoing paragraphs, I have outlined the more significant factors concerned in the production of the histologic picture which the pathologist sees under the microscope and attempts to interpret. The implications to be derived from this analysis will be better brought out after I have given the results of my experiences in the grading of tumors.

As stated before, the validity of the grading of a tumor rests on the confirmation of the prognosis from the section by the subsequent history of the patient.

I therefore first graded the 100 specimens according to their relative apparent malignancy and then compared the prognoses with the actual outcomes. Naturally, the end-results were not known to me from the follow-up records until the classification had been completed. In the grading, small cell size, much mitosis and little degeneration were taken as unfavorable. In the architecture, streaks, small nests, large nests, sheets and mucoid and adeno types were taken as of

9. Ingleby, Helen: Anatomic Study of a Case of Carcinoma of the Breast Giving Details of This Process, Arch. Path. **8**:653, 1929.

decreasing order of malignancy. Excessive lymphatic thrombosis was recorded. Vascular thrombosis was considered unfavorable.

After careful analysis, I concluded that the group could be divided into three classes: The first, or grade 1, should be dead in twelve months; the second, or grade 2, should be dead in twenty-four months, and the third, or grade 3, should be alive and well. The relation between predictions and actual outcomes may be summarized as follows: Class 1—correct 6 times, incorrect 9 times. Class 2—correct 35 times, incorrect 31 times. Class 3—correct 9 times, incorrect 10 times. Total—correct 50 times, incorrect 50 times.

It therefore seems as if the histologic criteria commonly used for the prognosis of relative malignancy are inadequate.

Not satisfied with this result because of the insistence of various workers on the validity of tumor grading, I tried another line of attack. In my 100 cases, seventeen of the patients had remained living and well. I therefore took the seventeen slides of the specimens from these living patients, mixed them with seventeen slides from patients who had died of metastases within eight months of operation, and attempted to tell which was which. My efforts to distinguish the two sets resulted in 54 per cent correct, and 46 per cent incorrect, guesses.

Not wishing to rely on my own judgment entirely, I asked and obtained the willing assistance of four experienced pathologists of Philadelphia in doing the same experiment. The highest score was 57 per cent correct, and 43 per cent incorrect, guesses. Mathematically speaking, since the guesses were 48, 52, 53, 54 and 57 per cent correct, the average correctness was 53 per cent, which is 3 per cent above the 50 per cent corresponding to pure chance, a dangerously narrow margin, certainly too small for practical use from the point of view of the individual patient.

Since in this group of 100 patients, seventeen were living and well, four were living with recurrences and presumably would soon die and sixty-one had died of metastases, in any random selection of slides (representing patients as they come for operation) the chances are 4 in 5 for selecting the slide of a patient who will die. If I use my judgment (i. e., examine the slide chosen at random and grade it), the chance of my being correct either way is slightly better than 50-50, i. e., pure chance.

As a matter of interest, I cite three specific cases.

Case 1.—The radical operation for carcinoma of the breast was performed by Dr. John B. Deaver on Mrs. F. S. in 1921, five weeks after the discovery of a lump in the breast. There was no clinical or pathologic axillary involvement. The tumor was about 1 inch (2.5 cm.) from the nipple, in the lower outer quadrant, fairly superficial, in a fatty breast. It was of the spherical type with no visible crablike prolongations, and only 1.5 cm. in diameter. The cells stained

well, appeared "differentiated" and had a tendency toward "adeno" formation, with few mitoses. No reaction was present in the stroma. An excellent prognosis! The patient remained perfectly well for fifty-five months; then several dozen tiny, shotlike nodules appeared on either side of the length of the scar on the chest wall, extending outward in both directions for about 6 inches (15.24 cm.). These nodules grew at about the same rate at first, until a number lower down on the chest wall, where there was considerable tissue between the skin and the ribs, soon outstripped the others. Above, where a minimum of subcutaneous tissue intervened between the skin and the ribs, the rate of growth of the nodules was much less rapid. Intensive treatment with x-rays and radium made no impression on the growth. Seventy months after the operation the patient died of loss of liver function from replacement by carcinoma.

Were the breast nodules a new tumor, or a number of different new tumors, or a growth of fragments remaining from the old tumor?

Histologic sections of four nodules showed identity as to carcinoma but dissimilarity as to detail. Several were so like the original, removed five years before, that without the labels it was impossible to separate them. They could be divided about as follows: The first was like the original growth. The second showed much more infiltration of lymphocytes, plasma cells, polyblasts, etc., but there was considerable necrobiosis of the smaller, more deeply stained cells. In the third, the cells were larger with more cytoplasm. They were arranged in fairly large nests, closely packed and growing into fatty areolar tissue. In the fourth specimen, some cells grew in smaller nests, but mostly they appeared in thin streaks through the interstices of a denser connective tissue, i. e., scar. The cells themselves were smaller and denser, and contained few mitoses.

Histologically, it was not a new tumor, or a number of different new tumors, but a recurrence of the original growth; i. e., at the time of operation, the tumor already existed beyond the lines of incision for amputation.

What determined the differences in the rate of growth of the separate nodules? Both the gross and the microscopic appearances suggested strongly that the environment was the significant factor. The tumors in the looser tissue grew the faster.

Evidence consistent with the idea of environment as a determining factor is also present in the next case.

Case 2.—The radical operation for carcinoma of the breast was performed on Mrs. H. in 1923. The tumor was of the crablike infiltrating type. The breast itself was fatty. In some heavy bands of connective tissue, the tumor was composed of thin streaks and little nests of small, compressed, dark-staining cells; 0.5 cm. beyond, the tumor grew into the fat of the breast in larger nests, which were close together, with the cells larger and better stained, and having nuclei larger with more mitoses. Here, not only the architecture but also the cellular detail was influenced by the environment. The prognosis from the histologic section was bad, and was correct; for the patient died shortly after of metastases.

The third case illustrates the inability to prognosticate the extent and distribution of tumor fragments from the microscopic examination of the specimen removed.

Case 3.—The radical operation for removal of carcinoma of the breast was performed on Mrs. H. in 1924. All the data, including the histologic picture of the growth removed, pointed to a good prognosis. One month later she returned

complaining of "lumps" all over her body. The palpating hand found literally hundreds of small, shotlike, subcutaneous nodules everywhere in the arms, legs, back, abdomen, chest, neck and scalp. She died two months later of what might in full justice be termed "general carcinoma." All the nodules grew (but naturally not at the same rate). Certainly, this "accident" could not have been anticipated from the section of the tumor.

COMMENT AND CONCLUSIONS

It is obvious from cases 1, 2 and 3 that if one is to make a prognosis at all, the presence or absence and the situation of secondary deposits are important. Every experienced pathologist has seen the liver practically destroyed by metastases, the patient dying from loss of liver function without help from metastases elsewhere. Clearly, an accurate prognosis depends, also, on a prediction of where the tumor will go.

From the British reports and others,[10] there seems little doubt that the radical operation is attended with more favorable end-results; that the chances of survival are in inverse proportion to the length of time the tumor has existed, and that the presence of metastases is decidedly unfavorable.

As a matter of record, I am giving tables showing these relations in my 100 cases. They are too few for statistical analysis, but may be added to the data of others, so that a large total can be collected for final analysis at some future time. I will be glad to furnish any available details that are not included in the tables.

The facts recorded in this paper demonstrate what reason should make clear, that it is futile to attempt to decide, from an examination of a section of the tumor, what will happen to a patient with cancer.

In the first place, even if one agrees that the inherent capacity for growth differs from tumor to tumor, it must be acknowledged that the factors that condition this difference vary in different persons, not only in their relative, but also in their absolute, influence. One cannot escape the general biologic fact that the form of expression of heredity is molded by the bars of environment. A few of the many environmental variables affecting the form expressed by the carcinomatous growth have already been cited in previous paragraphs. These variables, both structural and physiologic, cannot be controlled, for they are predetermined by the organ and the organism in which the growth is taking place. Neither can they be anticipated, nor can their respective values in the total picture be allocated, for one never knows what place they

10. Rep. Brit. Ministry of Health, London, no. 28. Lane-Claypon, J. E.: Cancer of the Breast and Its Surgical Treatment, ibid., no. 32; Further Report on Cancer of the Breast, no. 34. Leeds: Late Results of Operation for Cancer of the Breast, ibid. Lee, B. J., and Stubenbord, J. G.: Clinical Index of Malignancy for Carcinoma of the Breast, Surg. Gynec. Obst. **47**:812, 1928.

TABLE 2.—*The Factor of Time in Cases of Patients Operated on for Carcinoma and Dying of Metastasis*

Ages	Time of Death, Months	Time Between Consultation and Operation	Ages	Time of Death, Months	Time Between Consultation and Operation
51	24	12 months	36	13	3 months
50	45	5 months	42	14	12 months
30	10	3 months	57	21	1 week
45	12	6 months	47	21	10 months
61	37	6 months	38	21	6 months
50	17	7 months	48	31	9 months
55	37	12 months	57	13	5 months
36	7	4 months	51	26	18 months
41	12	5 months	48	8	4 months
65	14	2 weeks	44	12	6 months
51	30	4 months	45	4	10 days
50	39	6 weeks	42	30	2 months
59	6	24 months	52	17	24 months
45	24	5 months	28	18	11 months
45	72	2 months	41	21	10 days
30	16	12 months	34	13	13 months
70	16	24 months	42	9	At once
52	20	12 months	48	6	3 months
35	69	5 weeks	50	36	2 months
31	9	3 months	41	54	3 months
46	9	3 months	76	4	20 years
55	14	4 months	50	23	1 week
45	4	7 months	38	22	3 months
46	29	30 months	36	6	5 months
45	69	5 months	43	28	3 months
52	20	1 month	58	4	24 months
39	10	9 months	36	6	36 months
39	15	5 months	48	15	At once
30	8	6 months	40	3	4 years
55	20	1 month	47	21	2 months
			42	13	6 months

TABLE 3.—*The Factor of Time in Cases of Patients Operated on for Carcinoma and Dying from Causes Other than the Carcinoma*

Ages	Time of Death, Months	Time Between Consultation and Operation	Ages	Time of Death, Months	Time Between Consultation and Operation
40	6	6 months	43	14	4 months
45	12	3 months	68	35	6 months
67	72	2 months	69	7	5 months
54	23	2 months	60	6	12 months
71	34	9 months	66	16	2 weeks
35	16	1 week	57	40	3 months
63	63	3 weeks	40	53	6 years
50	21	1 week	55	11	6 months
66	31	2 years	61	6	10 months

TABLE 4.—*Twelve Earliest Cases in Which Patients Died of Metastasis*

Time, Mos.	Age, Yrs.	Type of Operation	Interval Before Operation
3	40	Radical axillary dissection	4 years
4	76	Simple axillary dissection	20 years
4	58	Radical axillary dissection	2 years
4	45	Radical axillary dissection	10 days
4	45	Radical axillary dissection	7 months
6	36	Radical axillary dissection	5 months
6	48	Radical axillary dissection	3 months
6	50	Radical axillary dissection	2 years
6	38	Radical axillary dissection	36 years
7	36	Radical axillary dissection	4 months
8	48	Radical axillary dissection	4 months
8	30	Radical axillary dissection	6 months
	Average 46.5		

occupied in the particular case prior to the emergence of the malignant growth.

Therefore, since these variables are determining factors in the production of the histologic pictures of carcinomas, and since one can neither control them nor assign to them their relative values in the general scheme, it should be obvious that the inherent vitality of the malignant growth is effectually distorted, if not entirely masked, and cannot be gaged. Small wonder that attempts at prediction are 50 per cent right and 50 per cent wrong!

TABLE 5.—*Twelve Earliest Cases in Which Patients Are Living and Well* *

Age, Yrs.	Metastasis †	Type of Operation	Interval Before Operation
44	No	Radical axillary dissection	2 months
54	No	Radical axillary dissection	3 weeks
78	Yes	Radical axillary dissection	4 weeks
56	No	Radical axillary dissection	4 years
55	No	Radical axillary dissection	2 years
61	No	Radical axillary dissection	6 months
44	Yes	Radical axillary dissection	4 months
46	No	Excision	5 weeks
48	No	Radical axillary dissection	0 months
51	No	Excision	3 months
65	No	Excision axillary dissection	11 months
45	No	Excision axillary dissection	1 week
Average 54			

* The smallest interval between the operation and the time of writing of these well patients was six years, four months; the longest interval was nine years.

† Two cases presented metastases and ten presented none.

TABLE 6.—*Age Distribution of the 100 Patients from Whom Specimens of Carcinoma of the Breast Came*

Age Group	No. Patients
26 to 30	4
31 to 35	4
36 to 40	11
41 to 45	23
46 to 50	19
51 to 55	15
56 to 60	9
61 to 65	6
66 to 70	6
71 to 75	1
76 to 80	2
Total	100

Further, in histologic grading one attempts, by examination of the removed specimen, to determine how it grew, and then to transfer this decision as a prediction of what will happen to any possible fragments left behind by the surgeon.

Is it not more accurate to determine by gross examination how a tumor grew? The patient says it was present for a certain length of time. How much did it grow in that time? Of course, one cannot know how long a tumor (in the breast) has existed. One can only hope to find out how long the patient has noticed it before she comes for consultation. But here sharp distinctions of time are not always

made. Most patients speak in round numbers, saying 6 months or 12 months rather than 5, 7, 11 or 13 months. This important fact is discussed and curves are given to illustrate it by Safford and me.[11] Naturally, one does not measure the rate of growth of a tumor, for, as a conscientious physician, one takes it out instantly.

If a malignant tumor is local for a certain length of time, however long or short, the operation should remove all the tumor and there is no need of grading it. But if a malignant tumor is general from the very beginning (as I think it is not) it is obvious that the variables are so complicated that one can see whether or not it recurs only by watching the patient, as has been done from the dawn of medicine.

TABLE 7.—*Operations*

Outcome	Radical	Simple Amputation	Dissection of Axilla Simple Amputation	Removal of Tumor
Dead from metastases	52	1	7	2
Dead from other causes	14	3	1	0
Living, with recurrence	3	0	0	1
Living and well	9	0	3	4
Totals	78	4	11	7

TABLE 8.—*Axillary Involvement* *

Outcome	Positive, Clinically and Pathologically	Negative, Clinically and Pathologically	Clinically Positive, Pathologically Negative	Clinically Negative, Pathologically Positive
Living and well	0	4	3	5
Dead of carcinoma	31	11	3	14
Living, with recurrence	1	0	0	2
Dead of other causes	8	3	1	4

* Axillary nodes were not removed in 10 cases.

The mass of evidence is, of course, in favor of the theory that tumors begin as local growths, without any general involvement. Unfortunately, the majority of patients are operated on when the growth is no longer local enough for complete removal.

The vital question is "Will the tumor come back?" It is clear that the answer does not lie in the microscopic but in the gross aspects. Did the surgeon leave any behind? If he did, the tumor will reappear from these fragments—unless they perish. And experience gives but little hope of this. If, on the other hand, the tumor is completely removed, there is no possibility of its return. It is all in a jar of

11. Reimann, S. P., and Safford, F. H.: Statistical Study of the Influence of the Educational Campaign on the Interval Between Discovery and Consultation in Mammary Carcinoma, Rep. Internat. Conference on Cancer, London, 1928, p. 562.

formaldehyde. In either case, attempts at grading the tumor are a waste of time. If in a patient from whom every vestige of the original growth was removed, another does develop, it must be a new one. Thereupon, I presume, one starts all over again and grades the new one. If some fragments have been left behind, one does not need to determine from the histologic preparation that the tumor will recur. It is known that it will. The only possible thing one can hope to grade is the rate at which the tumor will recur. The idea boils down simply to an attempt at guessing the rate of growth of unknown numbers and sizes of fragments in unknown positions left behind from a tumor which was partly removed surgically. And this cannot be done.

The question naturally arises, If fragments are left behind when a tumor is removed, why do they not immediately continue to grow instead of waiting for years as they often do (case 1)? Why do they grow at all? The answer to these questions is contained in the solution of the entire problem of cancer, which will not be had until a knowledge is obtained of the chemical basis of the regulation of cell proliferation. This has been intensively studied in this institute by Hammett, and in the succeeding paper he will put his results into a workable and investigatable hypothesis expressed in definite chemical terms.

SUMMARY

An attempt to grade the relative malignancy of 100 cases of cancer of the breast from the histologic picture of specimens obtained at operation gave false results, as determined by the later history of the patients from the follow-up records. A discussion of the biologic basis of this outcome, together with an inquiry into the rationale of the histologic grading of tumors, has been made. The conclusion is that the grading of a tumor for the individual patient is impossible at present.

Laboratory Methods and Technical Notes

UNTREATED HUMAN BONE SECTIONED WITH NEW KNIFE*

ARCH HIRAM MORRELL, M.D., NEW YORK

The delay in cutting sections of bone for microscopic study which is occasioned by the necessity for first decalcifying the bone is well known. It occurred to me that this might be obviated by using a knife of such hardness that it would cut sections of untreated bone. For this purpose a microtome knife was made from an alloy of tungsten carbide and cobalt which is manufactured under the trade name of "Widia." With this, sections varying from 6 to 12 microns in thickness have been cut from bone immediately after its removal during an operation and without any preliminary treatment. Other similar sections were cut from extremely dense undecalcified bone which had been fixed in a solution of formaldehyde.

In view of the fact that at present physicians have practically no knowledge of the chemical forms existing in living bone, whether colloid combinations or highly complex molecules of organic and inorganic substances, it is believed that the microscopic study and microchemical analysis of bone can now be approached in a manner heretofore impossible, with greatly increased possibilities of successful study.

420 East Fifty-Ninth Street.

* Submitted for publication, Oct. 22, 1929.

* From the Pathological Laboratory, New York Orthopaedic Dispensary and Hospital.

General Review

METHODS FOR THE HISTOLOGIC STUDY OF NORMAL AND DISEASED BONE*

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NEW YORK

CONTENTS

Many methods have been developed for the study of bone because the various details of structure cannot be demonstrated by any single method. A survey of the methods may be found in Schaffer's[1] article in the Encylopedia of Histological Technique and in Schmorl's,[2]

* Submitted for publication, Dec. 10, 1928.

* From the Laboratory Division of the Hospital for Joint Diseases.

1. Schaffer, J., in Krause: Enzyklopaedie der mikroskopische Technik, Berlin and Wien., Urban and Schwarzenberg, 1926, vol. 2, p. 1148.

2. Schmorl: Die Pathologisch-Histologischen Untersuchungsmethoden, ed. 12, Leipsic, F. C. W. Vogel, 1922.

Mallory and Wright's [3] and Lee's [4] textbooks. In Schaffer's article, practically every method described to the year 1926 is covered. Many of the methods are critically surveyed and his personal observations, after about thirty years' work in the field, are given.

The approach to the problem of technic in the study of bone becomes easier when one considers that there are two main structural elements in bone—fibrils and cells. The fact that the fibrils are resistant to injury while the cells are easily injured or destroyed, and the fact that most of the methods for the demonstration of the fibrillar structure will not demonstrate the cells and their processes, show the necessity of approaching the study of bone with these facts in mind. Further, it is important, in selecting the methods for the study of bone, to differentiate between methods for an examination for diagnosis and those for a demonstration of structure.

The greater number of procedures for the study of bone deal with its preparation for normal histologic study. Formerly, such studies were made mostly on ground disks, the preparation of which the older histologists developed to a high degree of technical perfection. Sections are, of course, now used almost exclusively and, as I shall point out later, the sections demonstrate practically everything that ground disks do. To review the entire literature of methods for bone is unnecessary, but I shall discuss some of the more important problems in the preparation of bone for study and give some useful directions for staining.

DECALCIFICATION

In order to make sections of bone, it must be decalcified, and this is the foremost difficulty in preparing bone for study by staining. Nothing new has been added to the principles of decalcification for fifty years. No decalcifying fluid has been compounded that will decalcify hard bone without either injuring its nuclear structure or damaging its fibrils. Many weak organic and strong inorganic acids have been tried, with the result that, for the most part, only a few decalcifying fluids are used, though these are in many respects unsatisfactory. Animal bones seem to withstand decalcification better than human bones; this may be due to the difference in the compactness of the bone. In general, it may be said that mineral acids possess the greatest decalcifying power, but are most injurious to the nuclei, while the organic acids produce the greatest swelling of the fibrils but less injury of the nuclei, if the exposure to the acid is not too prolonged.

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3. Mallory and Wright: Pathological Technique, ed. 8, Philadelphia, W. B. Saunders Company, 1924.

4. Lee: Vade-Mecum, ed. 8, Philadelphia, P. Blakiston's Son & Company, 1924.

Fixation of Bones That Are to Be Decalcified.—As a rule, only well fixed material should be decalcified. Fixation in strong alcohol, mercuric chloride, saturated aqueous picric acid, Zenker's solution and Flemming's solution does not prevent the swelling of the collagenous fibers, but formaldehyde does prevent it. For studying adult bone, the most satisfactory fixative is a 10 per cent neutral aqueous solution of solution of formaldehyde; even for embryonal bone, it is frequently necessary to use formaldehyde when certain staining methods are to be carried out.

The greatest number of the fixing solutions are weak acids or acid mixtures. They are therefore capable of acting on embryonal or poorly calcified bone as fixing and decalcifying solutions simultaneously. However, such solutions are useful only for small pieces of tissue. Zenker's or other acetic acid mixtures are of practical value for use with slightly calcified tissue.

Decalcification After Embedding in Celloidin.—Small, delicate tissues in which relationships must be preserved, as that in the internal ear, the cranium of small animals or zoologic objects, may be embedded in celloidin before decalcification. When the celloidin blocks are of proper consistency and air free, they are placed in water until they sink, and then they may be decalcified in aqueous acid mixtures, care being taken to secure quick decalcification by allowing free contact between block and acid. After the decalcification, the celloidin blocks are placed in 5 per cent lithium or sodium sulphate for twenty-four hours. They are then washed in running water for the same length of time. The blocks are transferred at length into alcohol, the strength of which is increased to 85 per cent. During this treatment, the celloidin does not lose any of its consistency or transparency.

Decalcification of Dry, Macerated Bone.—Dry, macerated bone should be placed in water or in a 0.5 per cent solution of sodium chloride for several hours before decalcification.

General Directions for Preparation and Decalcification.—Tissues to be decalcified should be cut into thin blocks, preferably under 2 mm. in thickness. This may be done with a fine jeweler's saw or, if the bone is soft, with a sharp knife or razor. If the latter is used bone dust does not enter between the trabeculae.

In addition to the right choice of a decalcifying fluid, it is of value in decalcification to use large amounts of the decalcifying agent, to agitate the fluid frequently, to decalcify at 37 C., to change the fluid frequently, to test the specimen and remove it as soon as decalcification is achieved and completely to remove all traces of the decalcifying agent before further treatment of the tissue. The progress of decalcification should be tested with a needle rather than with a nail or by bending the tissue.

Complete critical discussions of decalcifying mediums and conclusions are given by Busch,[5] Haug,[6] Schaffer,[7] Ziegler [8] and Lee.[4]

In the laboratory of the Hospital for Joint Diseases, New York, my associates and I have been constantly confronted with the problem of finding the proper decalcifying fluid to be used under given circumstances, for the fluid that should be used varies with the purpose of the examination and the character of the tissue to be examined. I shall describe in detail the use of the more important decalcifying fluids, and point out the pitfalls encountered in their use. The less important decalcifying fluids will be summarized.

Decalcifying Agents in Use.—Mueller's Fluid: This fluid is excellent for decalcifying fetal bones, if they are not too large. Mueller's solution has also been found useful for decalcifying spongy human bone (small pieces up to 2 cm. with thin cortex), bones of guinea-pig and rat, and pathologic human bone from osteomyelitis and tuberculosis. Large pieces of cortical bone or other compact bone could not be cut after the use of Mueller's solution. The sodium sulphate in Mueller's original solution is superfluous; a 2.5 to 5 per cent aqueous solution of potassium bichromate acts just as well. Decalcification may be hastened by using large amounts of the fluid, by keeping the tissues in an incubator at 37 C., by changing the decalcifying fluid at least twice a week and by stirring the solution daily. Mueller's is supposed to act as a fixative at the same time, but Haug denies that it does. It is advisable to fix bone tissues in formaldehyde before decalcifying them in Mueller's solution. After decalcification, the bones must be washed in running water for at least twenty-four hours.

The great disadvantage is that it takes weeks to decalcify spongy bone. During the long process of decalcification with Mueller's solution, there is a progressive hardening of the tissue, but I have found this action of the bichromate to be an advantage rather than a disadvantage when the bone was not too compact originally, for the bone is more easily cut after being embedded in paraffin. The nuclei of the cells may be injured if the tissue remains in Mueller's solution for too long a time, becoming pyknotic; but this is not a serious handicap, except when the finer nuclear structure of the bone cells is being studied. The distinction between dead and living bone remains, for in dead bone there is little, if any, nuclear staining. The fibrillar staining may be interfered with owing to the prolonged action of the potassium bichromate. The use of

5. Busch: Arch. f. mikr. Anat. **14**:480, 1877.

6. Haug: Ztschr. f. wissensch. Mikr. **8**:1, 1891.

7. Schaffer, J.: Ztschr. f. wissensch. Mikr. **19**:359, 1902.

8. Ziegler, P.: Festschr. zum siebenzigsten Geburtstag von Carl v. Kupffer, Jena, 1899.

Mueller's solution for decalcifying was developed by Pommer, who found it particularly useful for differentiating osteoid tissue from real bone.

The slow action of Mueller's fluid may be improved by combining it with nitric acid 0.5 to 1 per cent. Schmorl [2] recommended 3 per cent nitric acid. However, unless the bone is compact, nitric acid should not be resorted to. For giving a generally stained picture of bone for diagnostic purposes it has proved to be the best decalcifying fluid in my experience.

Nitric Acid: Nitric acid is effective for large pieces of bone and it is conceded to be the best decalcifying agent among the strong acids. It cannot be used for the study of fibrils examined by the polarizing microscope. It causes little swelling of the tissue when used in a 5 per cent solution. In other solutions, the degree of swelling of the tissue that it will cause depends on the concentration of the acid. Busch observed that prolonged treatment in a dilute acid was more injurious to the tissue than a short stay in a strong acid. Nitric acid has been used in watery solution in strengths of from 1 to 10 per cent. Many have recommended its use with substances that prevent swelling, such as chromic acid, mercuric chloride and phloroglucin. Schaffer is of the opinion that the addition of such substances to the watery solution of nitric acid is not only unnecessary but to a great degree injurious to the tissues. Recently, Küster, Schmorl, Preiswerk and others have also discarded the use of such substances. There is experimental proof that a solution of 5 per cent nitric acid made up in 95 per cent alcohol is only one-fifth as effective as the 5 per cent aqueous solution. Furthermore, nitric acid dissolved in diluted alcohol shrinks and thickens the collagenous fibers.

As for decalcification with nitric acid, it needs only the 5 per cent watery nitric acid followed by the essential treatment in 5 per cent lithium or sodium sulphate for twenty-four hours, during which the fluid is changed until it gives a neutral reaction with litmus, and washing for from twenty-four to forty-eight hours in running water. The use of much stronger solutions hastens decalcification only slightly and may injure the tissues severely. The tissues should be well fixed and should be placed in the acid immediately after the fixative has been washed out by water. Agitation of the tissue while in the decalcifying fluid reduces the time necessary for its decalcification. Decalcification is much hastened if the container is placed in a mechanical shaking machine. If the tissue is not agitated, its surface becomes coated with bubbles of gas which impede the action of the acid. If the pieces are large or compact, the acid should be changed several times, particularly in the beginning.

Some maintain that human bones may remain in nitric acid for four days without suffering serious injury. However, the tissues must remain in the acid only as long as is necessary for their complete decalcification; for, in my own experience, the stainability of the nuclei is greatly impaired if the tissues remain in the acid for one day. While nitric acid is recommended, when a strong acid must be used for tissues to be subsequently stained, nevertheless, I cannot endorse it as wholeheartedly as Schaffer and Mallory do for human bones.

If the nitric acid destroys the nuclei, it is then often impossible to differentiate between living and dead bone.

Hydrochloric Acid: Hydrochloric acid quickly and effectively decalcifies large pieces of bone, but it destroys the nuclei. Therefore, it can be recommended only for the demonstration of fibrillar structures of bone; for this it is unexcelled. In dilute solutions, hydrochloric acid causes more swelling than in concentrated solutions and, to prevent swelling of the tissues, it, too, has been used in combination with many substances such as chromic acid, phloroglucin and alcohol.

The method originated by von Ebner [9] in 1874 gives excellent results in the preservation of the fibrils and lamellar structures. Romeis [10] and Petersen [11] recently corroborated the value of this fluid for such studies. Large amounts of the fluid should be used, and it should be changed frequently. Petersen used concentrated hydrochloric acid made up as a 5 per cent solution in 10 per cent sodium chloride. After decalcification, the bone is placed in a 10 per cent solution of sodium chloride, to which small amounts of an alkali, such as lithium carbonate, are added until the solution is neutral. The tissue remains in the neutralized solution of sodium chloride for forty-eight hours, after which the bone is washed in running water for twenty-four hours and dehydrated in the usual way. Before decalcification, good fixation is necessary and fixation in a 10 per cent neutral solution of solution of formaldehyde or 95 per cent alcohol is the most desirable. This is the method used when the fibrillar structure is to be studied by polarized light.

Less Important Decalcifying Fluids.—Formic Acid: This produces the greatest swelling of the collagenous tissues, and may be used for decalcification of bone only after the bone has been fixed in formaldehyde. The addition of chromic acid or gold chloride moderates the swelling. Schaffer, using formic acid, specific gravity 1.2, was able to decalcify a section of human tooth from 1 to 1.5 mm. thick in twenty-four hours. The tissue should be transferred directly into strong alcohol after decalcification.

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9. Von Ebner: Sitzungsb. d. k. Akad. d. Wissensch. **71-72**:49, 1875.
10. Romeis: Naturwissenschaften **10**:733, 1922.
11. Petersen: Methoden zum Studium der Knochens, Ztschr. f. wissensch. Mikr. **43**:355, 1926.

Chromic Acid and Chromic Acid Mixtures: Pure chromic acid has a slight decalcifying action. It is used best in a 1 per cent solution for tissue containing little calcium. Its greatest usefulness is in being added to other acids to prevent swelling, as for instance when it is used as 0.5 per cent chromic acid in 1 to 2 per cent nitric acid.[12] For delicate objects, the following is recommended: 1 per cent osmic acid 10 cc., 1 per cent chromic acid 25 cc. and water 65 cc. After decalcification, the tissues are placed in 70 per cent alcohol in the dark.

Acetic Acid: According to Schaffer,[7] acetic acid causes much swelling of the tissues when used alone. A mixture of equal parts of saturated aqueous solution of sodium chloride (about 30 per cent) and concentrated acetic acid (50 per cent) decalcifies slowly but prevents swelling and shrinking. If the acetic acid is combined with osmic acid or gold chloride, or if the tissues have first been fixed in formaldehyde, the swelling is also lessened. In combination with bichromate, it is used as Zenker's fluid by some for decalcification of small pieces of bone.

Lactic Acid: Though Haug[6] and Ziegler[8] reported lactic acid satisfactory when used in 10 per cent solution, Busch and Schaffer found that it caused marked swelling of the tissues.

Phosphoric Acid: Phosphoric acid was also used in 10 and 15 per cent solutions and found, on the whole, unsatisfactory.

Citric Acid: Citric acid acts like phosphoric acid, but injures the cells.

Picric Acid: In saturated watery solutions, picric acid is good for use in the preparation of small fragments of bone, and has been used in combination with 3 to 5 per cent nitric acid for larger bones.

Sulphuric Acid: In 5 per cent aqueous solution, sulphuric acid is a good decalcifying agent for large pieces of bone, but it causes swelling of the tissues. The swelling recedes after the washing. It does not offer any advantage over nitric acid and it causes precipitates to form in the tissues, which, however, may be washed out with water.

Trichloracetic Acid: In 5 per cent aqueous solution, trichloracetic acid acts quickly, causes much swelling and necessitates treating the bone with alcohol directly after the decalcification. It does not have any special advantages.

Sodium Citrate: Recently, a 20 per cent solution of sodium citrate in a 10 per cent solution of solution of formaldehyde was recommended as a rapid and satisfactory decalcifying agent. I found that with bones of normal rats it acted more slowly than Mueller's fluid.[13]

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12. Ranvier: Traité technique d'histologie, Paris, F. Savy, 1889. Gebhardt: Arch. f. Entwcklngsmechn. d. Organ. **10**:137, 1900.

13. Shelling, D. H., and Halperson, May B.: A Rapid Method for Decalcification, Arch. Path. **5**:835 (May) 1928.

THE PREPARATION OF DISKS AND SECTIONS

General Procedure.—Bone may be prepared for histologic examination by being decalcified or it may be studied without decalcification. In the latter case, ground disks are made of fresh (fixed or unfixed) or macerated bone, or tiny fragments are scraped from the bone with a scalpel and studied. The disks or fragments may be examined unstained with the polarizing microscope, or stained or unstained with the ordinary microscope. The decalcified bone may be cut with a freezing microtome or may be embedded either in celloidin or in paraffin. Paraffin and celloidin sections are stained, and frozen sections are examined either stained or unstained. This is the general procedure for decalcified bone.

As to the superiority of the celloidin over the paraffin preparations in the study of bone there is doubt. I have used both and prefer paraffin preparations for all but extremely large sections. If Florida cedar wood oil is used for clearing the tissues instead of xylene, which makes the tissues brittle, and if the tissues are not permitted to remain for more than three hours in the paraffin oven, good sections will be obtained, provided a sharp knife is used. After decalcification, bone may be handled in the same way as any other tissue, except that care must be taken lest stained sections of bone that have been cleared with xylene, which renders them brittle, do not fall off the side.

The Preparation of Undecalcified Bone.—Fragments and Hand Cut Sections: Tiny fragments of normal or pathologic bone from which the soft tissues have been scraped with a scalpel may be examined directly under the microscope by placing them in a solution of sodium chloride. Glycerin should not be used.

The spongy bone of younger animals, as, for instance, the condyles of the femur of the young rabbit, may be easily cut with a sharp knife, and preparations may be obtained which may be examined directly under the higher power magnifications with or without staining.

Ground Disks: For the preparation of large cross sections or longitudinal sections of bone without decalcification, the grinding methods must be used. There are many methods of preparation, the disks being prepared with files, grindstones or grinding powders. On the whole, the powders are considered unsatisfactory. The choice between grindstones, files and powders is one of individual preference. The file method, while possibly a little more difficult, is conceded to give the best disks.

If disks are to be prepared from dry bones without soft parts, the bones must be carefully macerated and completely defatted. Disks may also be prepared from fixed or from fresh bone. Exact directions for the maceration of the bone are given by Ranvier.[12] The bone, which

should be fairly fresh, is separated from all its soft parts, the marrow cavity is opened, but the bone is not permitted to dry. The bones are then placed in water and kept in a warm place for several weeks or months. They are then brushed under a forceful stream of water and placed in the air to dry, but not in the direct sunlight. They must become completely white. If they show yellowish transparent areas, it indicates that they still contain fat and they must be defatted by treatment with benzene, toluene or xylene at 37 C. The disks may now be ground.

Gebhardt,[12] who is acknowledged to be highly competent with the disk method, gave the following directions: After the bone is macerated, it is placed in a vise and cut with a fine hand saw, the section being thick enough so that it does not bend. One surface of the section is ground with a file until it is even and the filings are removed with a stiff brush. The bone is then smoothed and polished by rubbing on frosted glass. Some thick, warm Canada balsam is placed on a glass slide, and the piece of bone with the ground surface downward is pressed with the fingers against the slide until the balsam hardens, care being taken that the balsam does not become brittle. Then the other surface of the bone is ground with files of increasing fineness until the finishing point is reached, at which the disk is transparent. Gebhardt frequently obtained sections that were less than 30 microns in thickness. The filings are removed by a stiff brush. Then this side of the bone is polished on a plate of frosted glass. The surface is then covered with warm balsam and mounted under a coverslip. Gebhardt usually prepared small disks, rarely over 2 or 3 cm. in the largest dimension.

For fine objects that might be injured in the grinding or polishing, or when preparations of bone and its soft parts are wanted, the bone should be embedded entirely in Canada balsam. Sections are then cut with the saw and ground. In Reinicke's [14] method, thick Canada balsam is warmed in an iron vessel, and the tissue is placed in it. Together they are warmed carefully and slowly. If the heating is too fast, the Canada balsam becomes brittle and the preparation will be unsatisfactory. The balsam is tested from time to time with a needle; if the balsam remaining on the needle hardens quickly and does not become indented by the finger-nail, then it is thick enough. The bone is removed, the balsam is permitted to dry and the bone is cut into sections with a saw. One surface is smoothed and the disk is glued with balsam to a clean, warm slide and ground. Care must be taken that air bubbles do not exist between the block and the tissue. The grinding and polishing are done as described.

14. Reinicke: Beitr. z. neueren Mikr., 1860, vol. 2, p. 57.

The preparation of moist disks of fresh bone or bone fixed in alcohol was first recommended by Volkmann [15] and also used by Matschinsky [16] and Gebhardt.[12] These disks are prepared in the same way as those of macerated bone. The advantage of using fresh or fixed bone is that the bone may be stained after the disks are prepared. Volkmann preferred making disks of fixed or fresh bone because he felt that he could demonstrate certain pathologic changes of the bone ground substance, as for instance granular cloudiness, molecular disintegration and the appearance of calcium granules, which, according to him, are shown only by this method.

Preparation of Decalcified Bone.—Decalcified bone may be embedded in celloidin or paraffin and studied in stained sections, as stated. It may also be cut on the freezing microtome, and the frozen sections may be examined either stained or unstained. Unstained frozen sections are, in practically every way, as good or better than ground disks, and they may be substituted for ground disks, except when the calcium of the ground substance is the object of the investigation. Petersen recently emphasized the usefulness of this procedure. My own experience justifies considerable enthusiasm. I have examined frozen sections under the polarizing microscope and have obtained pictures in every way as good as those presented in Gebhardt's article. I have seen disks that were distinctly inferior to frozen sections, although they were prepared at much greater effort. The simplicity of the preparation of frozen sections in itself is a distinct advantage. The best frozen sections are obtained from cortical bone, sections of spongy bone sometimes falling apart.

To secure good frozen sections of spongy bone, the trabeculae must be supported by some solid medium. Pieces of spongy bone are infiltrated with a 12.5 per cent aqueous solution of gelatin for four hours or longer in the incubator at 37 C. and then for an equal period in a 25 per cent solution. The entire container is then removed from the incubator. The gelatin is allowed to harden, and is then covered with 10 per cent solution of solution of formaldehyde. Before being frozen, the block must be trimmed and washed in running water for about an hour. Gelatin blocks must be over-frozen and then allowed to warm up a bit before cutting, as the inside is hard to freeze. Good sections are obtainable. I do not know of any way to remove the gelatin without spoiling the section. However, the gelatin does not interfere with examination of the sections under polarized light or of the stained sections.

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15. Volkmann: Arch. f. klin. Chir. **4**:437, 1863.
16. Matschinsky: Arch. f. mikr. Anat. **39**:151, 1892.

THE FURTHER TREATMENT OF DISKS AND SECTIONS OF BONE

When disks or sections of bone are prepared, one is ready to study the structure of bone. One can study (1) the ground substance, which includes the fibrils, cement lines, Sharpey's fibers, elastic fibers and the inorganic salts; (2) cellular elements, which include the osteoblasts and the bone cells with their processes; (3) the architecture of the bone; (4) the growth or epiphyseal regions; (5) the zones of preparatory ossification of normal bone and the so-called osteoid tissue of pathologic bone.

Demonstration of Structures in the Ground Substance of Bone.—The fibrils of bone may be shown in ground disks, frozen sections or stained sections. The first and still classic studies on the fibrillar structure of bone were done by von Ebner in 1874.[9] Gebhardt,[17] in studying the fibrillar structure of bone, used only disks, prepared, for the most part, from fresh bone by the method described. He pointed out that von Ebner's fluid, if the bone is to be decalcified, is the best solution to use, for it avoids injury of the fibrils and permits examination of the bone with the polarizing microscope. Bone that has been treated with phenol or phenol derivatives cannot be used for polarizing work, because the phenol changes the refractive index of the collagenous fibers. For the study of fibrils, Gebhardt mounted his disks in balsam and examined them with the polarizing microscope.

Weidenreich[18] studied the fibrillar and lamellar structure of bone using a modification of the Matschinsky silver method on disks of macerated bone. The disks prepared from macerated bone are washed in distilled water, and then placed in a 1 per cent solution of silver nitrate, in which they remain for twenty-four hours in the dark. After being washed with distilled water, they are placed in 10 per cent solution of solution of formaldehyde for one hour at 50 C. The disks stain dark brown. After the washing in water, it is necessary to grind the bases of the disk on a file or slate stone until the surface precipitate is removed. The disks are washed in water and then placed for about six hours in a 5 per cent solution of sodium thiosulphate and then for twelve hours in running water. Then they are dehydrated in alcohol, and embedded in Canada balsam. I impregnated the piece of bone first, then attached it to a slide and ground it down with a file and embedded the disk in balsam. To demonstrate the fibrillar structure better, instead of placing the disks for one hour in 10 per cent solution of solution of formaldehyde at 50 C., Weidenreich placed the disks in the same solu-

17. Gebhardt: Arch. f. Entwcklngsmechn. d. Organ. **20**:199, 1905-1906.

18. Weidenreich: Knochenstudien: 1. Tiel, Ztschr. f. d. ges. Anat., abstr. 1, Ztschr. f. Anat. u. Entwcklngsgesch. **69**:382, 1923.

tion for twenty-four hours at room temperature and then treated the disks in exactly the same way.

Weidenreich said that many times he got excellent results by these methods but that the results were not uniform. He then made use of the Weigert fibrin method, which he found satisfactory.

Weidenreich [18] modified Weigert's fibrin method in regard to differentiation and used this method successfully to demonstrate the fibrillar structure of bone and the cement lines. Five per cent nitric acid was used for decalcification and the tissues were embedded in paraffin. The method used is the same as that given in Mallory and Wright's [8] book on pathologic technic, eighth edition, page 191 up to step 7, or in Schmorl's [2] book, editions 12 and 13, to step 5, on page 146. The only modification is in the differentiation, which he carried out as follows: After the sections have been washed in water and blotted, they are differentiated in a mixture of three parts xylene to one part aniline oil, then two parts xylene to one part aniline oil, then one part xylene to one part aniline oil. The aniline oil must be of a high grade. As differentiation progresses, stain is extracted more slowly, and it is necessary to leave the sections in the differentiating fluid longer. Continued control with the microscope is necessary. Differentiation must be carried on until a stain is not given off, and fibers appear sharply in the ground substance, which is almost completely decolorized. The sections must then be well cleared in xylene and mounted in balsam. If the nuclei are to be stained, it must be done before the fibrils are stained. After trying various carmine solutions, I found that staining with lithium carmine for about five minutes and differentiating with acid alcohol gives a clear red nuclear stain, which stands out nicely in contrast to the blue fibril stain. I have used this method on embryonal and adult lamellar bone. The results, despite every care in differentiation, are not uniformly good. Coarse-fibered embryonal bone gives better results than adult lamellar bone.

This method is supposed not to be applicable after fixation or mordanting of tissue in fluids containing chrome salts. The best fixative is supposed to be alcohol. After the treatment with chrome salts, the sections should be treated before staining for from one-half to one hour with a 0.33 per cent solution of potassium permanganate, then washed in water and treated with 5 per cent oxalic acid for from two to three hours. After being thoroughly washed, they may be stained. After Zenker fixation, such preliminary treatment has been found unnecessary. Excellent results have been obtained using Weigert's original method with or without the modification, by controlling the degree of differentiation under the microscope.

Studnicka [19] used Bielschowsky's method for bone fibrils, fixing the bone in formaldehyde and decalcifying it in nitric acid. Directions for the subsequent treatment may be found in any standard textbook. Using this method, I have obtained some strikingly beautiful preparations, especially of the cement lines and general architecture of cortical bone. However, the results were not by any means uniform. The chances of success are greatest when thin frozen sections are used.

The fibrils were studied by Petersen [11] in frozen sections of bone examined with the polarizing microscope. I have found this to be the simplest method of demonstrating fibrils. The bone is fixed in formaldehyde solution and decalcified in von Ebner's fluid as described under the head of decalcification. Then pieces are cut on the freezing microtome, the sections being from 40 to 50 microns in thickness. Thicker sections give better pictures of the perforating canals and the architecture of the substantia spongiosa. These sections are studied unstained. Unstained preparations for the polarizing microscope give the best pictures by Petersen's method when mounted in watery solutions. They are mounted in a medium such as 5 per cent chloral hydrate, 10 per cent sodium chloride with 5 per cent chloral hydrate, or a mixture of 10 per cent sodium chloride and 10 per cent calcium chloride with 5 per cent chloral hydrate. The sections must be fat-free. If fat is present, rapid transference from water through alcohol to xylene and through alcohol back into water removes it completely. Wrinkles must not be considered, because in water the tissue becomes smooth. Remnants of periosteum must be carefully removed with a soft brush and all smudges cleaned away from the glassware, for these things interfere with the examination under the polarizing microscope. Air bubbles must be carefully avoided, for they attach themselves to the section. Finally, the cover glasses must be sealed with wax colophonium cement, more wax than colophonium, so that it is viscid and not brittle. The wax colophonium cement is prepared as follows: 500 Gm. of colophonium is melted over a low flame, and 125 Gm. of yellow bee's wax, 200 Gm. of yellow ochre and from 3 to 5 Gm. of linseed oil are added, the mixture being all the while stirred well. The mixture is just allowed to come to a boil and is then removed from the flame. It may be poured into paper molds of convenient size and allowed to harden. Before use for sealing, it is melted slowly. Large slides and cover glasses that extend beyond the section are indispensable. The preparations last long, but it is necessary that the cement be in good condition.

To demonstrate the bone fibrils under the polarizing microscope, the sections may be treated with phosphotungstic acid, which does not greatly change the double refraction. The sections may be enclosed

19. Studnicka: Ztschr. f. wissensch. Mikr. **23**:414, 1906.

directly in 5 per cent phosphotungstic acid, or may be left in this solution for from twelve to twenty-four hours and then enclosed in a solution of chloral hydrate. I have found that this treatment is not essential.

Sharpey's Fibers: Formerly, Sharpey's fibers were demonstrated by isolation or in disks, but more recently they have been demonstrated in sections. It is important to use the proper material, for while Sharpey's fibers may be demonstrated rather consistently in the fiber bone of the primary fetal skeleton they are rarely seen in adult cortical bone, and then only in the ground lamellae. There are numerous methods given in Schmorl's textbook and in Schaffer's review, including chemical processes, and staining and impregnation methods. Most of them are not consistently successful, and it seems that with the proper material Weidenreich's modification of Weigert's fibrin stain is the best.

Elastic Fibers: The methods for staining sections of bone for the study of elastic fibers are the same as for staining soft tissue, and again Weigert's fibrin stain can be recommended. Von Ebner advises the use of fresh tissue only, if disks are used; in macerated bone the elastic fibers may be dissolved. Chemical methods are employed, such as boiling a piece of bone for a short time in sodium hydroxide or boiling for a day in water. Koelliker boiled sections in acetic, oxalic or hydrochloric acid or else he removed the elastic fibers with cold potassium or sodium hydroxide. Elastic fibers are demonstrated best in the periosteum.

Cement Lines: To demonstrate the cement lines, frozen sections of bone decalcified according to von Ebner's method may be stained with Bielschowsky's silver stain. These structures are shown best in cortical bone.

Calcium: The calcium of the ground substance may be demonstrated in disks or sections either by staining methods or by chemical methods. For the microscopic demonstration of calcium in the ossifying skeleton, Schuscik [20] used embryonal bone fixed in alcohol, if the bones were to be embedded in celloidin, and in 10 per cent solution of solution of formaldehyde for fifteen minutes, if frozen sections were to be cut. She reported that all other fixatives and even water decalcify bone.

The surest way of demonstrating that a tissue contains calcium without locating it in the section is through the formation of crystals of plaster of Paris. Undecalcified sections are (1) placed in 40 per cent alcohol, (2) then on a slide, and (3) a drop of 2.5 to 3 per cent sulphuric acid is placed on the edge of the section: crystals of plaster of Paris appear quickly.

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20. Schuscik: Ztschr. f. wissensch. Mikr. **37**:215, 1920.

In none of the methods of staining for calcium, with the exception of the method of von Kóssa, in which silver nitrate is used, is there exclusion of the staining of the organic ground substance. Since all such methods stain calcium-free Müller preparations positively, only von Kóssa's method can be used without confusion.

The silver method of von Kóssa is as follows: (1) The bone is fixed in alcohol or for a very short time in formaldehyde, and then is washed in alcohol; (2) frozen, paraffin or celloidin sections (as preferred) are cut; (3) the sections are stained for from thirty to sixty minutes, in sunlight, in a 1 to 5 per cent solution of silver nitrate; (4) then they are washed in distilled water; (5) the excess silver salt is removed by placing the section in 5 per cent sodium hyposulphate; (6) the sections are washed in distilled water, and (7) counterstained with safranin, dehydrated and mounted in balsam.

The sections show yellowish staining of the calcified areas, which soon become black. Von Kóssa's method stains both calcium carbonate and calcium phosphate and is not selective for the latter. Even when this method is negative for calcium, the plaster of Paris method with sulphuric acid may be positive. Stains for staining calcium phosphate selectively have not been found.

The Cellular Structure of Bone.—The two kinds of cells with which one is concerned in the study of bone are the osteoblasts and the bone cells. To demonstrate the osteoblasts or the bone cells without their processes in a gross way is a simple matter. In any fixed preparation, they may be stained with the usual dyes, provided that the decalcifying agent, if one has been used, has not destroyed them. All the strong inorganic acids are destructive of the cells and their nuclei but nitric acid is, perhaps, of them all, the least destructive. This must be kept in mind particularly when diagnosis is concerned, for on the stainability of the nucleus depends the decision as to whether the bone is living or dead. If one is interested in studying the cells of the bone, a strong acid should not be used. But decalcification for a long time in Mueller's solution also injures the cells, so that it is evident that any minute cytologic studies on bone must be done on bone that is quickly decalcified or that does not need decalcification.

Bone Cells: One of the oldest and simplest methods of study of bone cells uses fresh, thin bone plates that have been deprived of periosteum. These are stained in carmine and mounted in glycerin. The nuclei and the protoplasm of the bone cells stain but not the processes. Satisfactory pictures of bone cells are obtained when thin pieces of young embryonal bone are fixed in Zenker's solution and sections of the undecalcified bone are stained with Delafield's hematoxylin and congo red or eosin. In such preparations, a few short protoplasmic processes may be demonstrated. Beautiful pictures are obtained when embryonal

or young infant bone is decalcified in Mueller's solution and stained by Mallory's eosin and methylene blue (methylthionine chloride, U. S. P.) method.

Bone Cell Processes: The problem becomes more difficult when, in addition to the cells, the processes are to be demonstrated. Bast [21] fixed extremely thin plates of periosteum-covered mammalian bone in 95 per cent alcohol, placed them in water and stained them from eight to twenty-four hours in a very weak aqueous solution of gentian violet. He dehydrated them as quickly as possible, placed the bone plates in benzene or xylene and then removed the periosteum completely. He mounted the bone plates in warm, thick, neutral balsam. With this method, the bone cells and their processes appear deeply stained; the older the bone, the clearer the staining. They are best seen under oil immersion. To prevent the stain from coming out, the absolute alcohol should be removed completely by several changes of benzene. I have found that Bast's method clearly demonstrates bone cells and their processes, but it is useful only when extremely thin plates of bone are obtainable; for example, the skull and nasal bones of young or embryonal animals. I was unable to use this method on the long bones of either young or embryonal animals because thin sections covered with periosteum were not obtainable.

Zachariades [22] demonstrated the processes of bone cells in the canaliculi in sections of freshly fixed fetal bone decalcified with acid and cut by hand or on the freezing microtome. According to his directions, a section is placed on a slide and treated for a few seconds with 1 per cent osmic acid for the fixation of the protoplasmic net. It is then washed and stained quickly in one drop of watery saturated solution of safranin. Then the section is covered with a few drops of 40 per cent potassium hydroxide and is slightly warmed until it becomes flat. The excess of potassium hydroxide is removed, and the section is covered with glycerin water. If the treatment with potassium hydroxide has not been too severe, the lacunae and the canaliculi of the bone may be seen, and, in addition, protoplasmic processes may be seen entering the canaliculi. Few processes are seen as compared with the number of canaliculi.

The best way to demonstrate the protoplasmic processes of the bone cells is as follows.[23] A well polished disk of fresh bone is made. The disk is stained for twenty-four hours in aqueous quinolin blue solution (a few drops of the alcoholic solution in 10 cc. of distilled water). The precipitate is removed with a brush before mounting. The protoplasmic processes are seen in the canaliculi, and they stain red. These

21. Bast: Am. J. Anat. **19**:139 and 321, 1921.
22. Zachariades: Ztschr. f. wissensch. Mikr. **10**:447, 1893.
23. Zachariades: Compt. rend. Soc. de biol. **1**:207, 1889.

processes are so delicate in lamellar bone that there may be some difficulty in seeing them. In fiber bone, they are easily demonstrable.

The demonstration of the bone cells and their processes in paraffin or celloidin sections by the gold impregnation and chrome silver method of Golgi [24] has been suggested, but these methods are, on the whole, not reliable, as pointed out by Schmorl.

Lacunae and Calaliculi: While the bone cells and their processes are difficult to demonstrate, the lacunae and the canaliculi in which they are enclosed in the ground substance are easily demonstrable. The walls of the lacunae are seen in ordinary sections stained with hematoxylin and eosin. The lacunae and the anastomosing canaliculi have always been demonstrated in unstained dried disks mounted in balsam or unmounted. I have been demonstrating the lacunae and canaliculi in frozen sections of acid decalcified bone by thoroughly drying the frozen sections, which should be rather thick, on smooth filter paper and mounting rapidly in glycerin or balsam. I prefer the glycerin. The lacunae and the thick network of anastomosing canaliculi stand out black and distinct on a transparent colorless background. While the results are as good as any of the results obtained by any method pictured in the literature, they are only temporary, rarely lasting for more than four or five days. The reason for this is that the method depends on the presence of air in the lacunae and canaliculi, but after a few days the air that was present in the dried section is replaced by the mounting medium, whether it is balsam or glycerin. I have not seen this method mentioned anywhere in the literature. It is simple and effective.

Finer Cytologic Details: In addition, several other details have been studied particularly in the osteoblasts of embryonal bone. These are the mitochondria, the Golgi apparatus, the secretion spheres of the protoplasm and the basophilic nature of the protoplasm.

For the demonstration of mitochondria in osteoblasts, Dubreuil [25] fixed thin plates of perichondral and endochondral embryonal bone from near the epiphysis in a mixture of bichromate and formaldehyde solution for from two to three days, then for eight days in 3 per cent potassium bichromate. After being washed, the bone was embedded in celloidin, decalcified in 5 per cent nitric acid, as described under decalcification, and stained with Heidenhain's iron hematoxylin. Deineka [26] also gave a good method.

For the demonstration of the secretion spheres in the protoplasm of osteoblasts as described by Dubreuil, embryonal bone is fixed in Lenhos-

24. Joseph: Arch. f. mikr. Anat. **6**:182, 1870. Bouin: Bibliog. Anat. **4**:207, 1896.

25. Dubreuil: Ann. d'anat. micro. **15**:53, 1913.

26. Deineka: Anat. Anz. **46**:97, 1914.

sék's mercuric chloride acetic acid mixture [27] for from six to twelve hours, then in 3 per cent potassium bichromate for a few days, and paraffin or celloidin sections are stained in iron hematoxylin.

For the demonstration of basophilic protoplasm of the osteoblasts, M. Askanazy [28] recommended staining sections of embryonal bone in concentrated watery solutions of Loeffler's methylene blue, or Unna's polychrome methylene blue. Differentiate in alcohol or aniline alcohol (1:10). If the bone also is to be stained, the following procedure is recommended: (1) stain the bone for from five to ten minutes in methylene blue, (2) wash it in water and (3) place it for from two to five minutes in 95 per cent alcohol plus two parts of saturated alcoholic eosin solution.

The General Architecture of Bone.—In studying the general structures of lamellar bone, a piece of dense cortex of a tubular bone should be examined. Any of the methods given for fibrils serves also for this purpose. Frozen sections of bone decalcified by von Ebner's method, and mounted in 5 per cent chloral hydrate, examined with an ordinary microscope, yield most satisfactory pictures when the light is cut down. Frozen sections that have been blotted dry may be examined under a coverslip without a mounting medium. A good stain for the general architecture of the bone is gallein, as described by Petersen. One tenth of a gram of powdered gallein is added to 100 cc. of boiling 5 per cent aluminum chloride and boiled for fifteen minutes. It is made up to volume and filtered, and paraffin, celloidin or frozen sections are stained for from twelve to twenty-four hours or more in a solution of one part stain to ten parts water. In my own experience with the stain, the dilute solution of gallein prescribed by him gave poor results, but with a half strength solution informative pictures were obtained after staining for from twenty-four to forty-eight hours. This may be due to the difference between European and American gallein. After being stained, the sections are washed in water to remove the excess stain and covered with 95 per cent alcohol, the excess being allowed to run off on filter paper. They are either preserved in 96 per cent alcohol or further dehydrated in absolute alcohol and cleared in xylene and mounted in balsam. Before being cleared and mounted, the sections may be treated with a 5 per cent solution of phosphotungstic acid for twenty-four hours, which makes the fibrils stand out especially well.

The Epiphyseal Region of Bone.—Endochondral Ossification: To demonstrate endochondral ossification, bone and cartilage must be dif-

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27. Lenhossék's fixative is made up as follows: saturated aqueous solution of sublimate, 75 cc.; glacial acetic acid, 5 cc., and 50 per cent alcohol, 25 cc., with saturation of the mixture with picric acid.

28. Askanazy: Zentralbl. f. path. Anat. **13**:369, 1902.

ferentiated. Generally, I use a double stain that is basic for the cartilage and acid for the bone. The staining of cartilage depends on the treatment it has had. Mueller's fluid dissolves and extracts the chondroitin-sulphuric acid and the cartilage does not give the characteristic stain with the basic dyes, but with Mueller's fluid it is possible to differentiate sharply the calcified from the uncalcified cartilage.

According to Schaffer,[1] page 1191, Escher fixes his material in picric mercuric chloride, decalcifies in alcoholic hydrochloric acid, treats sections with concentrated Delafield's hematoxylin for three minutes and then with alcoholic borax carmine for twenty-four hours. He then puts the sections in alcoholic orange G for a short time, clears them in carbolxylene and mounts them directly in balsam. The bone appears red, the cartilage blue.

In studying embryonal bone, I stained tissue decalcified by Mueller's method with Mallory's eosin and methylene blue method: with this procedure the bone and osteoid tissue stain red and the cartilage matrix stains blue, the intensity varying directly with the amount of calcium in the ground substance.

Preparatory Ossification Zones and Osteoid Tissue.—To demonstrate previously calcified areas in decalcified bone, Pommer recommended using Mueller's fluid in the case of embryonal bones after the first half of embryonal life. In such bones, not completely decalcified with Mueller's fluid, a clear difference is seen between the pale red osteoid substance and the intense red finished bone, when Delafield's hematoxylin and eosin are used.

The aniline dyes used by Pommer are more differential. His method is based on the belief that Mueller's solution does not entirely remove the calcium from the bone; it carries decalcification on to the stage in which bone can be cut. Pommer emphasized that decalcification with Mueller's fluid should be stopped when the tissue may be cut with a razor with the resistance of hard wood. Because Mueller's fluid does not decalcify completely, the bone containing calcium is supposed to be distinguishable from the bone that does not contain calcium (osteoid tissue) by special staining methods. When dilute ammonia carmine is used (see Schmorl[2] 12th and 13th editions, pages 109 and 239) the osteoid and completely decalcified tissues stain yellow to red, while the bone containing calcium, when hematoxylin is used in combination with carmine, stains blue. The use of Mueller's solution for distinguishing the so-called osteoid tissue from the bone containing calcium is best applicable in the case of pathologic bone such as that seen in osteomalacia, rickets and the fibrous osteodystrophias.

Recently, Bock[29] described a hematoxylin method for distinguishing osteoid tissue, useful for tissues decalcified in von Ebner's fluid or nitric

29. Bock: Ztschr. f. wissensch. Mikr. **40**:318, 1923.

acid. Bone is placed for from one to four weeks in a solution of four parts 10 per cent solution of solution of formaldehyde and one part Mueller's fluid. Decalcification in von Ebner's solution or nitric acid is followed by neutralization and washing of the tissues in the usual manner. The author then preferred slow embedding in celloidin, though paraffin may be used. The method of staining as given by Bock is as follows: Sections are stained for from twelve to eighteen hours in Hansen's hematoxylin, which must be prepared fresh each time. It is made of three solutions: (1) 1 Gm. of hematoxylin dissolved in 10 cc. of absolute alcohol, (2) 20 Gm. of potassium alum dissolved in 200 cc. of distilled water and (3) 1 Gm. of potassium permanganate dissolved in 16 cc. of distilled water. Solutions 1 and 2 are mixed and 3 cc. of solution 3 is added. The mixture is boiled for one minute. When cool it is filtered and is then ready for use. Strongly overstained sections are differentiated in a mixture of equal parts of pure glycerin and glacial acetic acid for from five to twenty minutes or longer, and then washed in running water for an hour. Sections are counterstained for five minutes in alcoholic eosin, four parts eosin to 1,000 cc. of 95 per cent alcohol. They are dehydrated, cleared and mounted in Canada balsam.

By this method, the osteoid tissue stains red, while the bone containing calcium stains a deep blue. This method is recent. Confirmations of its usefulness have not appeared in the literature.

CONCLUSION

The methods given are all tried procedures that have been found useful in studying bone from all angles. The choice of a method for the study of bone is conditioned by the purpose of the examination. The pathologist is most handicapped in the study of bone since the diagnosis of a lesion in bone rests mainly on the cellular elements and these are the structures most injured by decalcifying agents. The pathologist frequently need not use compact bone to make a diagnosis so that he may not have to decalcify the tissue or may be able to use Mueller's fluid. However, if decalcification is necessary, rapid treatment with nitric acid, though not entirely satisfactory, is the best procedure. Histologists, studying the structure of bone, are not confronted by this difficulty.

CORRECTION

In the article by Dr. Stacy R. Mettier, entitled, "The Structural Changes of the Liver in Pernicious Anemia: A Contrast Between Relapse and Remission," in the August issue (ARCH. PATH. **8**:213, 1929), the word "hyperatrophy," in the first conclusion in the Summary on page 222 should read "hypertrophy."

Notes and News

University News, Promotions, Resignations, Appointments, Deaths.—William T. Belfield, pioneer in genito-urinary surgery and one of the first American students of bacteriology, has died at the age of 73. Dr. Belfield delivered the Cartwright Lectures in New York in 1883, which were republished in book form under the title, On the Relation of Micro-Organisms to Disease.

The American Society for the Control of Cancer, 25 West Forty-Third Street, New York, has announced that Robert B. Greenough, assistant professor of surgery, Harvard Medical School, has become the chairman of its board of directors, and that Clarence C. Little, director of the Jackson Memorial Laboratory, Mount Desert Island, Me., has been appointed its managing director.

The Institute of Pathology (Howard T. Karsner, director) at Western Reserve University, Cleveland, was dedicated with appropriate ceremonies on October 7. The dedicatory address was given by Henry R. Dean, professor of pathology in Cambridge University, England.

A Central Bureau for the Study of Tumors in Philadelphia.—This bureau has been organized by Dr. Joseph P. McFarland, who acts as its director without pay. The bureau was established by the aid of Dr. George M. Dorrance, who underwrites the expenses for the first year, and of Dr. Stanley P. Reinmann, who obtained the use of the former pathologic laboratory of the Lankenau Hospital for the use of the bureau. The bureau is a strictly independent enterprise which begins by undertaking (1) to collect microscopic specimens of all tumors treated in all the hospitals of Philadelphia and vicinity, together with full data regarding each; (2) to identify, classify and make this material available to all scholars desiring to use it for research or comparison; (3) to follow up each case of malignant tumor until the death of the patient; (4) to use the data and specimens for study and publication, and (5) to collect and maintain a library of books and pamphlets on tumors and an index to the literature. In addition, it is intended that the bureau shall concern itself with larger problems and with the collection of data that may throw a light on fundamental questions.

Membership in the bureau is free to all pathologists, surgeons and roentgenologists who deal with tumors, without other obligation than the contribution of such material and data as they may have which will be collected by messenger from the bureau. It is expected that the Philadelphia Health Council will undertake the follow-up of patients suffering from malignant tumors.

The bureau is not in any sense a private laboratory in which tumors are diagnosed on application. It offers its benefits to its members without cost and will be conducted in the interest of science.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

INFLUENCE OF EXPERIMENTAL HYPERTHYROIDISM ON GESTATION. M. M. KUNDE, J. CARLSON and T. PROUD, Am. J. Physiol. **88**:747, 1929.

In severe hyperthyroidism induced in cretin rabbits there was observed an apparent increase in the number of developing graafian follicles and primordial ova. In rabbits with severe induced hyperthyroidism, the processes of estrus, ovulation, fertilization, migration and implantation took place, but the young were seldom born, resorption of the fetus taking place usually during the latter two thirds of pregnancy.

H. E. EGGERS.

THE BLOOD PLASMA OF NORMAL AND PARATHYROIDECTOMIZED ALBINO RATS. W. R. TWEEDY and S. B. CHANDLER, Am. J. Physiol. **88**:754, 1929.

In normal white rats, on a standard diet, the blood calcium varied between 9.25 and 12.5 mg. per hundred cubic centimeters. This value was not altered by unilateral parathyroidectomy, but on removal of the second parathyroid there was a temporary drop below the low normal value. This abnormal level persisted for a few weeks only. The drop occurred suddenly in some cases, rather slowly in others. By the end of 100 days, there was a definite tendency of the blood calcium to rise, and by the end of from 200 to 300 days, it may have attained a value just below low normal. Parathyroidectomized rats are from two to three times as reactive to comparable amounts of parathyroid hormone as are normal animals.

H. E. EGGERS.

A NEW DEFICIENCY DISEASE PRODUCED BY THE RIGID EXCLUSION OF FAT FROM THE DIET. G. O. and M. M. BURR, J. Biol. Chem. **82**:345, 1929.

A new deficiency disease, involving caudal necrosis, has been observed in rats maintained on diets extremely low in fat. Although the nonsaponifiable and glycerol fractions of fat appear to be ineffective in curing the disease, it has been entirely cured following the addition of 2 per cent of fatty acid to the fat-free ration.

ARTHUR LOCKE.

RELATION OF OESTRUS HORMONE TO NYMPHOMANIA OR PERSISTENT ESTRUS OF COWS. W. FREI and E. LUTZ, Virchows Arch. f. path. Anat. **271**:572, 1929.

Frei and Lutz devote thirty-seven pages to a critical review of the literature relating to the estrus hormone. Of the various substances that have been described as having an estrus-producing action, they consider that only the follicle fluid or the cells of the follicle wall contain the true hormone. They doubt that true and complete estrus has been caused by some of the substances used. The occurrence of the hormone in the corpus luteum and placenta, as described by many, is paradoxic. The authors believe the corpus luteum absorbs, stores and renders inactive the hormone formed by the follicle. Nymphomania of cows is a condition of persistent sexual desire or estrus. Manual rupture through the vagina of an enlarged ovarian follicle sometimes overcomes the condition. The vaginal smear of nymphomanic cows contained epithelial cells and leukocytes, but no hornified epithelia. The authors conclude that nymphomania is due to the persistence of unruptured follicles in the ovary; since no corpora lutea are formed, the follicle hormone acts continuously. Nymphomania may go into anaphrodisia when the ovary becomes completely cystic or atrophic.

O. T. SCHULTZ.

EFFECT OF BLOCKAGE OF RETICULO-ENDOTHELIAL SYSTEM ON LEVEL OF BLOOD SUGAR. E. J. STERKIN and E. L. KERNER-POSCHENJAN, Ztschr. f. d. ges. exper. Med. **64**:311, 1929.

A single intravenous injection (dogs) of india ink, vitargol or collargol generally did not affect the level of the blood sugar. After chronic, intensive blockage of the reticulo-endothelial system with the substances named, no change of level in the blood sugar beyond normal variation was obtained. Splenectomy with chronic blockage likewise was without effect. (These results are contrary to experiments reported by F. Venulet, and abstracted in this journal.)

BALDUIN LUCKE.

EFFECT OF RAREFICATION OF THE AIR ON NUMBER OF ERYTHROCYTES AND HEMOGLOBIN CONTENT OF NORMAL AND OF SPLENECTOMIZED ANIMALS. G. GIANNINI, Ztschr. f. d. ges. exper. Med. **64**:431, 1929.

Experiments were undertaken to decide whether the increase in the number of erythrocytes due to rarefication of the air is absolute or relative. If the increase is absolute, a number of erythrocytes must rapidly be destroyed on the return to normal air pressure, since, shortly after, the person or the animal used in the experiment presents normal values. Rarefication of the air was accomplished in a Loewy chamber; rabbits, guinea-pigs and rats were used. The number of erythrocytes increased from 3,000,000 to 3,500,000 per cubic millimeter above the normal after an exposure of from seventy-two to thirty-six hours to rarefied air (300 to 350 mm. of mercury). Polychromatophilia, anisocytosis and poikilocytosis occurred. On a return to normal air pressure, the erythrocyte counts quickly regained a normal level (often within forty-eight hours). The increase in hemoglobin content lagged behind the increase in the number of erythrocytes in the rats and the guinea-pigs, but paralleled that in the rabbits. Conversely, after the return to normal pressure, the increase in hemoglobin content persisted for a somewhat longer period than did the increase in number of erythrocytes. Rarefied air brought about a decrease in color index in rats and guinea-pigs, but not in rabbits.

In splenectomized animals, rarefication of air led only to a slight increase in the red cell count, but to a relatively greater increase in hemoglobin (both absolute and relative). The color index rose above 1 and remained at this level even six hours after the exposure. Destruction of red cells following the return to normal air pressure after exposure to rarefied air manifested itself by bilirubinemia, which appeared within twenty-four hours following exposure.

BALDUIN LUCKE.

LEUKOCYTOSIS AFTER BRAIN PUNCTURE. G. ROSENOW, Ztschr. f. d. ges. exper. Med. **64**:452, 1929.

Brain puncture (in rabbits) involving the corpus stratum, the thalamus or the hypothalamic region promptly leads to neutrophilic leukocytosis. There is no relation between the degree of puncture fever and the leukocytic curve. Puncture of other regions of the brain does not induce leukocytosis.

BALDUIN LUCKE.

Pathologic Anatomy

THE MICROSCOPIC ELEMENTS IN BILE. EDWARD HOLLANDER, Am. J. M. Sc. **177**:371, 1929.

Microscopic examination of bile from the gallbladder and bile ducts reveals four elements diagnostic of pathologic states of the biliary tract: bile flocculi, intensely bile-stained debris, agminated cholesterol crystals and sandlike particles.

PEARL M. ZEEK.

PRIMARY MULTIPLE SARCOMATOSIS OF THE SKIN. W. C. HUEPER and B. B. BEESON, Arch. Dermat. & Syph. **19**:794, 1929.

The clinical course and postmortem examination of a patient with multiple sarcomatosis are reported. The authors believe the neoplasm to be mesodermal and to belong to the group of primary, multiple round cell sarcomas of the skin. The exact origin is doubtful. The dense accumulations of tumor cells around vessels and nerves suggest that these tissues were the primary foci.

FRANK M. COCHEMS.

OCCURRENCE OF ACCESSORY PARATHYROID GLANDS. W. L. A. WELLBROCK, J. A. M. A. **92**:1821, 1929.

The parathyroid glands found in this series were situated anywhere on the anterior surface, on the isthmus or on the lateral surface, and a few were embedded in the thyroid tissue, just within the edge of the thyroid gland. One or more parathyroid glands were found in 7.76 per cent of the 1,056 thyroid glands examined. These were all checked by microscopic examination. The parathyroid glands are finely granular, soft, yellowish brown, lenticular, spheroidal or pear-shaped structures, from 2 to 10 mm. in diameter. Parathyroid glands are often confused with accessory thyroid glands, lymph nodes, hemolymph glands and lobules of fat. This is the chief reason for the failure of transplants. Parathyroid glands were found in equal numbers on exophthalmic, adenomatous and colloid goiters. The largest gland on which a parathyroid gland was found was adenomatous, weighing 275 Gm.; the smallest was a hypertrophic parenchymatous gland weighing 8 Gm. In two cases, three parathyroid glands were found, in one of which the three were in a cluster. In three cases, two were found, and in two cases one was on each lobe of the thyroid gland. In only one case was there mild transitory tetany following thyroidectomy.

AUTHOR'S SUMMARY.

PERINEPHRITIC ABSCESS. A. H. PEACOCK, Surg. Gynec. & Obst. **48**:757, 1929.

Suppurative processes in the perirenal tissue were regarded as being of renal or extrarenal origin, and the usual methods of infection were considered. Twenty-one cases are reported by the author. In four of renal origin, the process was secondary to cystitis in one, and to prostatic hypertrophy in one, and in two it was due to calculi in the ureters. In three of ten cases the perinephritic abscess followed furuncles, in four the condition was associated with pregnancy, in one each it followed tonsillitis and appendiceal abscess, and in one it was concomitant with actinomycotic suppuration that resulted in perforation of the duodenum and colon with resultant invasion of the tissues about the right kidney. In the remaining seven patients the etiology could not be determined. Bacteriologic examination of the abscesses revealed staphylococci in fifteen, *bacillus coli* in two, streptococci and actinomyces in one, and in two the cultures were sterile. The urine in ten was sterile, but in seven *B. coli* occurred, staphylococci were found in three, and streptococci in one. In almost all patients the abscess resulted in bulging in the costovertebral angle, in the abdomen or in the region of Poupart's ligament.

RICHARD A. LIFVENDAHL.

CECAL DIVERTICULOSIS, WITH SPECIAL REFERENCE TO TRAUMATIC DIVERTICULI. L. A. GREENSFELDER and R. I. HILLER, Surg. Gynec. & Obst. **48**:786, 1929.

Diverticuli of the cecum may follow appendectomy as the result of eversion of the bowel between constricting bands of omentum, of traction by omental adhesions and of migration of purse-string suture material toward the lumen with subsequent defect in the wall, or the circular muscle may be injured with resultant weakness of the cecal wall; in other instances the insufficiency may be caused by intramural abscesses. The material studied was obtained from two cases in which the patients were operated on and from two more of a series of 400 autopsies. In this number

of necropsies twenty-three of the bodies had had their appendixes removed. In addition appendectomy was performed in thirteen dogs. In the six animals in which the purse-string suture method was done, the percentage of these types of complications was more frequent than in the other seven in which the ligature-drop procedure was used.

RICHARD A. LIFVENDAHL.

PULMONARY ASBESTOSIS. W. BURTON WOOD, Tubercle **10**:358, 1929.

The author reports on fifteen cases of pulmonary asbestosis, presenting some interesting roentgenograms as well as reviewing the chemical composition of asbestos fibers. He covers the pathology and symptoms as well as the physical signs, prognosis and duration of exposure to the dust. The article should be read in the original as it is not suitable for abstract.

H. J. CORPER.

THE CHANGES CAUSED BY TREATING GENERAL PARESIS WITH MALARIA. T. VON LEHOCZKY, Arch. f. Psychiat. **86**:443, 1929.

A study was made of twelve cases of general paresis in which ten of the patients were treated with malaria and two with milk injections. Both methods had the same effects. In evaluating the pathologic evidence, one must distinguish between those phenomena which belong to the "basic process" (general paresis) and those which may be the result of the inoculation. Another distinction is urged between the lasting and the transitory changes, both ectodermal and mesodermal. Among the lasting changes are classed: fibrous meningitis, destruction of ganglion cells and processes and the increase in glia (quantitative glia changes). The transitory changes are: the inflammatory infiltration, the pathologic modification of the ganglion cells (as distinguished from their destruction), the myelin degeneration and the qualitative glia changes. It is concluded that malaria treatment has an effect on the transitory changes only. This dictum is in sharp contrast with the observation of Strauessler and Koskinas. These workers postulated a "healing inflammation" and interpreted their results as indicating a shift from the "malignant" type of inflammation (plasma cells) toward the "benign" type (lymphocytes). Aside from this shift, they noticed an "allergic change" to the effect that the nonspecific paretic process gave way to a specific gummatous appearance. Lehoczky denies both these claims. Malaria works only on the mesodermal structures. Among the infiltrative elements many degenerative forms were found: karyorrhexis in the lymphocytes and vacuole degeneration in the plasma cells. W. Freeman claimed a restitution of the disturbed lamination of the cortex. This "conspicuous exaggeration of the theory of Straeussler and Koskinas" is energetically rejected.

A. A. LOW.

ABSCESS OF THE LUNG OF TEN YEARS' DURATION. E. SCHLÜTER, Centralbl. f. allg. Path. u. path. Anat. **45**:6, 1929.

A man, aged 56, died immediately after exploratory puncture of an empyema cavity which had drained occasionally since its formation ten years previously. At autopsy, the left upper pulmonary lobe was found transformed into a cavity containing about 600 cc. of creamy pus. The lining of this cavity was of flat epithelium except in some depressions and projections where it was cuboidal or cylindric; occasional zones of granulations indicated recent inflammation. No communications with bronchi were demonstrable, drainage having taken place from the lung into the empyema sac and from this through the fistula to the outside.

GEORGE RUKSTINAT.

CALCIFICATION OF THE VASA DEFERENTIA. W. DOPHEIDE, Centralbl. f. allg. Path. u. path Anat. **45**:39, 1929.

Calcification of the vasae deferentiae was found in two men, aged 80 and 77, respectively. In both the process was limited to the middle circular muscle layer

and was accompanied by a connective tissue increase. In one instance, the process involved the entire circumference and had a length of 2.5 cm.

GEORGE RUKSTINAT.

NEPHRITIC RETINITIS. R. HANSSEN, Klin. Monatsbl. f. Augenh. **82**:40, 1929.

Nephritic retinitis does not arise as the result of a primary disturbance of the blood vessels but is essentially a toxic inflammatory process. In cases of nephritic retinitis showing changes in the heart and blood vessels together with increased blood pressure but with little decrease in kidney function, it cannot be demonstrated that the blood pressure is the cause of the retinitis. It is more probable that in such cases the injurious toxins which have produced the changes in the vascular apparatus have attacked the eyes earlier than the kidneys. The apparent thinness of the retinal blood vessels is not due to a contraction of the vessels or to ischemia, but is rather to be explained as a part of general anemia. From the latter there results a swelling and cloudiness of the tissues of the optic papilla which surround the vessel walls causing the retinal vessels to be less visible. The cystoid spaces in the retina occurring in nephritic retinitis are not filled with a simple serous transudate but with an inflammatory exudate rich in albumin and containing a fibrin network, fat and cells.

CHARLES WEISS.

CONGENITAL ANOMALY OF THE HEART. H. GERSTMANN, Virchows Arch. f. path. Anat. **271**:1, 1929.

The author describes a heart obtained from a new-born, full-term, female infant. It consisted of two auricles and a large ventricle, from which the pulmonic artery arose. The latter united with the aorta at the open ductus arteriosus. Separated from the main ventricular cavity by a perforated septum was a small cavity from which the aorta arose. This septum was not the true interventricular septum, but the persistent aorticopulmonic crest. The anomaly is considered a type IV in the classification of Spitzer, but is not characteristic of this type in that detorsion had been carried somewhat further than is the rule for Spitzer's type IV.

O. T. SCHULTZ.

STENOSIS OF CONUS ARTERIOSUS AND PULMONIC ORIFICE. L. SKUBISZEWSKI, Virchows Arch. f. path. Anat. **271**:14, 1929.

A man, aged 54, had been under medical care for cardiac decompensation at various times since 1922. He had always been cyanotic. The clinical diagnosis was mitral stenosis. Death occurred suddenly. He had been free from edema for some time previous to death. The heart weighed 600 Gm., the hypertrophy being chiefly of the right ventricle. The root of the pulmonic artery appeared slightly narrower than normal. The lumen of the conus arteriosus was diminished to 1.2 cm. in diameter. The segments of the pulmonic valve were fused and formed a funnel-shaped structure with an opening 4 mm. in diameter. The subaortic portion of the ventricular septum was defective. The lungs received their main supply of blood through a branch given off from the left subclavian artery.

O. T. SCHULTZ.

RHACHISCHISIS WITH TRIPLICATION OF THE SPINAL CORD. R. ALTSCHUL, Virchows Arch. f. path. Anat. **271**:45, 1929.

The condition which the author reports occurred in a girl, aged 9 months, who had mongoloid characteristics and was psychically subnormal. Muscular movements and plantar and knee reflexes were normal. The arm reflexes could not be elicited. In the cervical region was a fluctuant mass which measured 22 cm. in cross diameter by 7 cm. in height. Pressure on the mass caused convulsive movements. At necropsy, the laminae of the last three cervical and first dorsal vertebrae were absent. The cavity of the mass seen externally was lined by dura and com-

municated with the subdural space in the region of the vertebral defect. In this region the spinal cord was divided longitudinally into three parts. The left portion was fairly complete, the right and middle portions being somewhat more rudimentary. Each portion was surrounded by leptomeninges. The three portions united again at the level of the fifth dorsal vertebra, from which point downward the cord was normal. The author believes that this is the only recorded instance of longitudinal division of the cord into three parts. The embryogenetic explanation is difficult. He believes that the anomaly was due to inrolling of the dorsal medullary plates, which process was complete on the left and led to the formation of a complete left cord. The process was less complete on the right side and subdivided the original right cord into two parts.

O. T. SCHULTZ.

OSTEOSCLEROTIC ANEMIA WITH RETICULO-ENDOTHELIAL PROLIFERATION. A. A. WASILJEFF, Virchows Arch. f. path. Anat. **271**:134, 1929.

A man, aged 30, had complained for five years of pain in the lower extremities along the bones, not associated with swelling of the joints. The bones of the upper extremities also became painful during the latter course of his illness. He had had hemorrhages from the nose and urinary tract. He entered the hospital in a moribund condition. He was undernourished and anemic. Hematologic examination revealed 1,200,000 erythrocytes, 35 per cent hemoglobin, 11,450 leukocytes and 28,000 platelets. Anisocytosis, poikilocytosis, normalblasts and myelocytes were noted. The spleen was moderately enlarged. In all the bones examined post mortem, the marrow was replaced by pale tissue, which on microscopic examination consisted of large, ovoid cells derived from the vascular endothelium. This tissue had replaced the hematopoietic tissue with the exception of small groups of lymphocytes. Large cells of similar type were present also in the liver and spleen, and in the lungs they occurred in the form of small groups in the septums outside the capillaries, their position suggesting an origin from adventitial cells. They were present in the bronchial lymph nodes, but not in the other lymph nodes. The cells were suggestive of Gaucher cells, but did not have the characteristic morphology or staining reactions. They contained inclusions which had the staining reaction of mucoprotein. Microchemical staining reactions for lipoids were negative. Myelopoiesis was present in the liver and spleen. The author interprets the case as a metabolic disturbance associated with proliferation of the reticulo-endothelial system, which had led to replacement of the hematopoietic tissue of the bone marrow, where the reticulo-endothelial proliferation was combined with fibrosis.

O. T. SCHULTZ.

HEMATOLOGIC EFFECTS OF EXPERIMENTAL BENZENE ADMINISTRATION. G. ORZECHOWSKI, Virchows Arch. f. path. Anat. **271**:191, 1929.

Subcutaneous administration of benzene in rabbits causes no change in the erythrocytes or platelets, only a slight change in the lymphocytes, decreases the coagulation time and has its most marked effect on the granulocytes, which are apparently specificially destroyed.

O. T. SCHULTZ.

CHANGES IN THE SPLEEN IN MALARIA. B. A. PHOTAKIS, Virchows Arch. f. path. Anat. **271**:192, 1929.

The author divides the cases of malaria in which he has been able to study the spleen post mortem into acute cases in which death was caused by malaria, chronic cases in which death was due to malaria and chronic cases in which death was due to some other cause. The acute cases are subdivided into those which run a short, hyperacute, highly toxic course with hyperpyrexia and extreme destruction of erythrocytes. In such cases the spleen may measure 40 by 20 by 18 cm. and weigh as much as 1,200 Gm. It is soft, brownish black and almost diffluent on section. The size is due chiefly to engorgement. The hyperplasia present is limited to the sinus endothelium, which exhibits marked phagocytosis of

injured red blood corpuscles. In a second group of acute cases the course is more prolonged, lasts from six to eight weeks and death is due to the malarial infection. In such cases the spleen is also greatly enlarged, is slightly firmer and is a grayish black or grayish red. In such spleens reticular hyperplasia is associated with sinus endothelial hyperplasia and predominates over the latter. In chronic cases the spleen shrinks and becomes firmer, and the chief hyperplastic change occurs in the connective tissue framework of the organ.

O. T. SCHULTZ.

PATHOLOGY OF SUPRARENAL. E. OMELSKYJ, Virchows Arch. f. path. Anat. **271**:377, 1929.

Kovács, of Vienna (*Beitr. z. path. Anat. u. z. allg. Path.* **79**:213, 1928), had described, in a patient with Addison's disease, a condition of bilateral disappearance of the suprarenal cortex, the medulla being spared. Kovács termed the condition cytotoxic cortical contraction of the suprarenal gland and held the pathologic changes of the suprarenal gland to be the result of some toxic substance which acted specifically over a long period of time on the cortical cells. The present author, stimulated apparently by Kovács and working under the direction of Erdheim, attempts to establish more firmly the conception of specific cytotoxic destruction of the suprarenal cortex. The chief basis of his paper of fifty-five pages is a case of hypophyseal cachexia, in which the suprarenal glands showed the kind of change described by Kovács. The case, described clinically and microscopically in great detail, is placed in the group termed multiple sclerosis of the endocrine organ by Falta. There were no symptoms of Addison's disease. The chief symptomatology was ascribed to loss of function of the hypophysis, which was found at necropsy to be small and fibrotic. Terminal evidences of myxedema were held to be due to the fibrotic thyroid, and the early menopause to premature sclerosis of the ovaries. The cortical injury in the suprarenals, which was systemic and not local in origin, occurred late; hence, definite evidences of Addison's disease were not present. By comparison of the microscopic changes of the suprarenals in this case with those of other forms of contraction of the suprarenal, the author attempts to establish histologic criteria which will permit differentiation of suprarenal atrophy of systemic origin, that is, cytotoxic contraction, from atrophy due to causes acting locally, such as infection, thrombosis and tuberculosis.

O. T. SCHULTZ.

ACUTE PLACENTITIS. W. LAUBSCHER, Virchows Arch. f. path. Anat. **271**:450, 1929.

The author reports a case of placental infection, followed by sepsis and death of the patient; it was possible to study both the placenta and the uterus in this case. In a second case only the placenta could be studied. In each case, rupture of the membranes had occurred early and labor was protracted. The organisms gained entry from below, penetrating at the margin of the placenta, and spreading beneath the fetal membrane surface. Freedom of the intervillous spaces from inflammatory reaction is evidence that the infection was not hematogenous in origin.

O. T. SCHULTZ.

HYPERTROPHIC PULMONARY OSTEOARTHROPATHY. C. CRUMP, Virchows Arch. f. path. Anat. **271**:467, 1929.

Crump, of Boston, presents from Erdheim's institute a detailed study of a case of hypertrophic pulmonary osteo-arthropathy, for which he prefers the name generalized osteophytosis, which is interesting because the case was closely followed clinically. The patient was a woman, aged 50, whose breast had been amputated for carcinoma in 1923. A year and a half later she returned to the hospital, where she remained for over a year. Fifteen months before death, following a slight, febrile pharyngitis, swelling of the dorsum of the foot and pain in the small joints of the foot, hand and fingers caused her to return to the hospital. In

time, the elbows and knees became painful. Involvement of the left lung was evident when she entered the hospital because of the joint pains; a roentgenologic diagnosis of secondary carcinoma of the lung was later made. Hypertrophy and osteophytosis of the bones was evident roentgenologically at examinations made eight and three months before death. The fingers became clubbed. The patient presented the three classic manifestations of the Bamberger-Marie syndrome, namely, hypertrophy and osteophytosis of the bone, involvement of the joints and clubbing of the fingers. Of these, the changes in the bone are the only ones which are characteristic. They may occur alone, but permit the diagnosis; the arthritis or clubbing of the fingers may occur alone, diagnosis being impossible, or either may be combined with the lesion of the bone. The microscopic changes of the bones are described in detail and are illustrated by numerous photomicrographs. The process is essentially one of subperiosteal bone formation, in the form of broader plates and of osteophytic excrescences. The process appears to be periodic, the new bone being laid down in lamellae or laminae. The bone is porous, but would probably become more condensed with longer duration of the disease. The involvement of the joints consists of a chronic arthritis and synovitis. Crump believes that the disease is due to a toxin of unknown nature, which is liberated in diseases of the lung and heart, more rarely of the liver or lymph nodes. The toxin may attack the bones, the joints and the soft tissues about the terminal phalanges of the fingers, or only one or two of these situations may be attacked. Four closely printed pages of bibliographic references conclude the article.

O. T. SCHULTZ.

CIRCULATORY SYSTEM OF DICEPHALIC MONSTER. OLGA SEAMON, Virchows Arch. f. path. Anat. **271**:512, 1929.

In the monstrosity studied, the heads were separate, the vertebral columns were separate down to the sacrum, the internal organs were duplicated with the exception of the urinary bladder and the two livers were fused. Especial attention was given to study of the circulatory system, since the heart of one-half was rudimentary and had no connection with the vascular system of its portion. The relations of the two circulatory systems are described in detail, and the embryogenesis of the cardiovascular anomaly is discussed.

O. T. SCHULTZ.

HISTOLOGY OF CHONDRODYSTROPHIC CHICK EMBRYOS. W. LANDAUER, Virchows Arch. f. path. Anat. **271**:534, 1929.

Histologic study of the endocrine organs of chondrodystrophic chicks, removed from the shell on the twenty-second day of incubation, failed to reveal any changes which might be correlated with the chondrodystrophic condition. The only change noted was somewhat delayed differentiation of all the endocrine organs, as compared with normal embryos of the same age.

HISTOLOGIC LESIONS IN EARLY DIFFUSE GLOMERULONEPHRITIS. R. HUECKEL, Virchows Arch. f. path. Anat. **271**:211, 1929.

The patient died thirty hours after the sudden onset of the disease. There were small, irregular, oblong, dark red spots on the cut surfaces of the kidneys. The brain was edematous. The circular edema around the vas afferens and the swelling and vacuolization of its walls were found only in a few glomeruli. They are considered secondary to the combined glomerulonephritic and glomerulonephrotic changes as presented by the kidneys in this unusually early case. They cannot be the first lesion in glomerulonephritis.

ALFRED PLAUT.

DEXTROVERSION, INVERSION AND TRANSPOSITION OF THE HEART. A. SPITZER, Virchows Arch. f. path. Anat. **271**:226, 1929.

Spitzer dedicates his seventy-eight page monograph to Professor Julius Tandler, the anatomist, in honor of the latter's sixtieth birthday. The article is based on a heart obtained from a boy who died at the age of 6 years and who had been

cyanotic from birth. The first third is a minutely detailed description of the changes found, the remainder consisting of a discussion of the factors involved in the genesis of the malformation. The latter appeared at first glance to be a simple dextroversion, but closer study revealed that both inversion and transposition were concerned in bringing about the anomaly. The heart had a large, hypertrophied right ventricle with, however, a typical mitral valve. The left ventricle was small and had a typical tricuspid valve. The ventricular septum was defective, the defect beginning in the subaortic region and part of the margin of the defect being formed by the aorticopulmonic crest. The ductus Botalli was patent, and the foramen ovale was open. The smaller left ventricle (transposed right) gave rise to the aorta, which was situated to the left, and the pulmonary artery, which was to the right and was atretic in its intramuscular or conus portion. The malformation is discussed as a possible atavistic reversion to a stage in ontogenetic or phylogenetic development, and the relation of dextroversion, inversion and transposition to each other and the possible combinations of these processes are elaborated in detail.

O. T. Schultz.

Uremic Dermatitis. R. Roessle, Virchows Arch. f. path. Anat. **271**:304, 1929.

Among twenty-nine autopsies of uremia cases, skin lesions were found in twenty-four. Kidney disease without uremia does not seem to make skin lesions. The skin lesions in uremia are not characteristic. Coats of lymphocytes around the blood vessels (upper layer) are found most frequently. Only in severe cases are the epidermis and the deeper layers of cutis affected. Swelling of endothelium occurs and the walls of capillaries may become indistinct. Leukocytes, plasma cells and mast cells were missing; eosinophilic leukocytes were found once. Small necroses occur in the connective tissue of the cutis. Degenerative changes in the epithelium (ballooning degeneration) were marked in the skin of an old painter, with extreme arteriosclerotic atrophy of the kidneys. This lesion is similar to herpes zoster, and it was in the skin of this same patient that perineural inflammation was found. The mechanism of the formation of the skin lesion is unknown since it is not known what uremia is. The amount of urea in the skin, however, was found fairly well in ratio to the degree of the skin lesion.

Alfred Plaut.

Reaction of Fixed Tissues to Infection. G. Meyer, Virchows Arch. f. path. Anat. **271**:317, 1929.

The literal translation of the title of this article is "Connective Tissue and Foreign Body." The foreign bodies, the effects of which were studied, were micro-organisms, chiefly staphylococci, which enter the skin by way of the hair follicles and caused furuncles. The reaction set up in the subdermal tissue is a progressive and continuing one, differing in this respect from that caused by nonliving foreign bodies. In the interpretation of the changes which occur, the conception of von Mellendorf is accepted. The supporting connective tissue is looked on as a syncytium or plasmodium, composed of living cells capable of reproduction and of fibrils which cannot reproduce themselves. The syncytium is transformed into a multipotent mesenchyme when its cells are stimulated to proliferation. The entrance of the foreign micro-organism causes degeneration and destruction of the nonreproducible collagenous and other fibrils. The cells, stimulated to amitotic division, give rise to multipotent mononucleated cells, which become transformed into lymphocytes, granular leukocytes and phagocytic histiocytes. These cells, the exudate cells supposedly derived from the blood stream according to the older conception of inflammation, are held to arise chiefly locally from the fixed tissue. A point which is emphasized is their origin by amitosis. Mitosis, when it occurs, as it does after the process is established, leads to new formation and regeneration of the fixed tissues, not to wandering cells.

O. T. Schultz.

EFFECT OF DIFFERENT KINDS OF COAL DUST ON THE LUNGS. H. BORCHARDT, Virchows Arch. f. path. Anat. **271**:366, 1929.

Belief that pigmentation of the lungs due to carbon particles results from inhaled material dates back to Pearson, in 1813. The doctrine of the inhalational pigmentation of the lungs was denied by Virchow, but was, of course, later established again as correct. Of the various kinds of foreign material which are inhaled, coal or carbon dust appears to be least irritating and to cause least reaction in the lung. Differences in the degree of reaction to coal dust, however, have been noted in different localities and have led to controversy as to the innocuousness of coal dust. Borchardt believed that physical and chemical differences in the inhaled coal might be responsible for differences in tissue reaction and put the matter to the experimental test by subjecting rabbits to the inhalation of dust-laden air for one hour daily. The materials used were soot, animal charcoal, hard coal or anthracite and soft coal. Microscopic examination revealed distinct differences in the degree of fibrosis, this being greatest after inhalation of soft coal dust, less after hard coal and slight after animal charcoal, with practically no reaction after soot. The degree of self-cleansing of the lung was in inverse order to the degree of tissue reaction. The differences noted in the lungs of the experimental animals were comparable to those seen in the lungs of human beings.

O. T. SCHULTZ.

TERATOMA OF THE HYPOPHYSEAL REGION WITH REFERENCE TO SUPRARENALS IN ANENCEPHALY. E. J. KRAUS, Virchows Arch. f. path. Anat. **271**:546, 1929.

An otherwise well formed prematurely born girl, 42 cm. long, had a teratoid tumor which protruded into the cavum cranii and into the pharynx; the canalis craniophyryngeus was wide. The brain was normal. The anterior lobe of hypophysis was found on the posterior aspect of the cranial part of the tumor; it was compressed but normal. Rathke's cysts and posterior lobe were absent. The suprarenal glands were normal. Therefore, one cannot assume that in anencephaly the defect of the suprarenal glands is due to absence of the posterior lobe of hypophysis.

ALFRED PLAUT.

MORPHOLOGY OF LYMPHADENOID TISSUE. J. WÄTJEN, Virchows Arch. f. path. Anat. **271**:556, 1929.

This is a contribution to the nature of the so-called germinal centers of the lymph nodes. Because of the lability of the lymphoid tissues it is necessary, in order to understand the normal variations, to study nodes from various regions of the same body, nodes at different age periods and nodes from persons in varying nutritional states. The author believes that the germinal center of Flemming or the secondary follicle of Heilmann is entirely connective tissue in origin, and that it results from atrophy or disappearance of lymphoid cells with their replacement by proliferated stroma cells. The author does not think it has been established that these cells can take on lymphoblastic properties.

O. T. SCHULTZ.

DOES THE CAPILLARY ENDOTHELIUM OF THE HYPOPHYSIS BELONG TO THE RETICULO-ENDOTHELIAL SYSTEM? GUSTAV SINCKE, Ztschr. f. d. ges. exper. Med. **63**:223, 1928.

Because of their common function of producing reticulum and of lining sinusoidal blood and lymph spaces, Aschoff grouped the capillary endothelium of the hypophysis with the reticulo-endothelial cells of the sinuses of the lymph nodes, the blood sinuses of the spleen, the capillaries of the liver (Kupffer's cells), the capillaries of the bone marrow and the suprarenal cortex. On the basis of an extensive series of experiments on rats (and in a few cases on rabbits) with fifteen different dyes and many other substances in the colloidal state, Sincke came

to the conclusion that the capillary endothelium of the rat should not be grouped with the cells of the reticulo-endothelial system in the restricted sense of the word. While the colloidal metals, carbon, arsphenamine, etc., were stored by the Kupffer cells and other reticulo-endothelial elements, the endothelial cells of the hypophysis did not generally take them up. Small quantities of some of the dyes were stored by the capillary endothelium of the pars intermedia.

DIFFUSE INTESTINE-LIKE ADENOMA OF THE RENAL PELVIS. ALFRED PLAUT, Ztschr. f. urol. Chir. **26**:562, 1929.

A woman, aged 57, developed a fistula and a swelling at the site of operation after removal of a kidney stone. The kidney consisted of a flabby cystic structure the walls of which were lined with what seemed to be large intestine at first sight. There are mucus-producing glands, and there is much lymphatic tissue and a considerable amount of muscle. Part of the muscle can be traced to the muscularis of large blood vessels. Few glomeruli are preserved, but there is a considerable number of mostly narrow tubuli. In spots "embryonic kidney blastema" is found. The piece of ureter which was removed with the kidney is also lined with mucus-producing glands, but there is no overgrowth of muscle in it. This tumor cannot be classified; it is different from the tumor-like lesions in ecstrophy of the bladder.

AUTHOR'S SUMMARY.

Microbiology and Parasitology

MORPHOLOGIC AND BIOLOGIC STUDIES OF THE SPECIES OF DIPHYLLOBOTHRIUM IN CHINA. ERNEST CARROLL FAUST, HORACE E. CAMPBELL and CLAUDE R. KELLOGG, Am. J. Hyg. **9**:560, 1929.

The literature on infections with *Diphyllobothrium* in the Orient, particularly with *Sparganum mansoni*, and on the experimental development of adults from spargana in man and other vertebrates is critically reviewed. The results of a study of material collected from various vertebrate hosts in China are presented. The following species, all from China or its environs, are described: *Diphyllobothrium decipiens, D. mansoni, D. ranarum, D. erinacei, D. houghtoni* n. sp. *D. okumurai* n. sp. *D. latum* and *D. cordatum.* A discussion of the general characteristics of the genus *Diphyllobothrium* is presented, in which it is found desirable to divide the genus into two subgenera, *Diphyllobothrium* sensu stricto and *Spirometra* n. nom., on the basis of a rosetted outer uterine mass and eggs with rounded ends in the former subgenus, and a spirally piled outer uterus and eggs with roundly pointed ends in the latter subgenus. The type species of the genus is designated as type of the subgenus *Diphyllobothrium,* while the species *decipiens* is designated as type for the subgenus *Spirometra.* The experimental evidence which is reviewed confirms the subgeneric division. The distribution of the several species of *Diphyllobothrium* is outlined. The pathogenicity of the species of *D. decipiens* and *D. erinacei* is briefly presented.

AUTHORS' SUMMARY.

A CASE OF SPLENOMEGALY SHOWING PARATYPHOID BACILLI. HENRY EDMUND MELENEY, Am. J. Trop. Med. **9**:97, 1929.

Under the name "tropical febrile splenomegaly" have been grouped a number of distinct diseases. One of these occurs outside the tropics, notably in North China in the neighborhood of Peking and Tientsin. A case of this kind is described in which a paratyphoid bacillus was isolated from the spleen at the time of splenectomy. Although the etiology in cases presenting this clinical picture may not always be the same, there is little doubt that it always involves a micro-organism the chief activity of which is in the spleen, with a secondary effect on the liver. Careful culturing of the blood of such patients, together with cultures of material from spleen and liver punctures, and of liver and spleen at the time of splenectomy, if carried out early in the disease, should yield important data on its etiology.

AUTHOR'S SUMMARY.

EPIDEMIOLOGY OF BLACKWATER FEVER IN SIAM. E. C. CORT, Am. J. Trop. Med. **9**:105, 1929.

Blackwater fever, before unknown in Siam, appeared in Chiengmai in epidemic form. The first epidemic was confined practically to one city block and to three family connections. The second epidemic occurred in a boy's school 4 miles from the city. A short time after the first epidemic, sporadic cases, twelve in all, began to appear. The evidence seems to point to a specific factor, supposedly a malarial parasite, but a variety capable of elaborating a potent hemolysin. Prevention of relapse in a series of cases with neoarsphenamine without quinine seems to suggest that this parasite is a variety of *Plasmodium vivax*. But it should be noted that these same observations from treatment alone might point to a spirochete or a leptospira.

AUTHOR'S SUMMARY.

PELLAGRA ASSOCIATED WITH ORGANIC DISEASE OF THE GASTRO-INTESTINAL TRACT. ROY H. TURNER, Am. J. Trop. Med. **9**:129, 1929.

The literature is briefly reviewed. The observations in a group of sixteen patients are presented in the form of a table. They all had pellagra and with it some organic disease of the gastro-intestinal tract. They represent 20 per cent of the patients studied. Symptoms of pellagra may obscure those of serious organic disease. The death rate in the group was high, 50 per cent dying in the hospital in spite of dietary treatment along most modern lines, as compared with 25 per cent in the control group. Two of the patients, one without diarrhea until after dermatitis had appeared, had been on an excellent diet throughout. The diet of four others was good up to the time the diarrhea appeared. The effect of such lesions on bacterial growth in the intestinal lumen and the absorption of harmful substances from the lumen is discussed. The suggestion is made that this may be an important factor in the production of the pellagra.

AUTHOR'S SUMMARY.

TUBERCULOUS LESIONS IN THE LUNG OF A NEGRO CHILD NINE WEEKS OLD. WILLIAM SNOW MILLER, Am. Rev. Tuberc. **19**:119, 1929.

A small tuberculous lymph node situated in the pleura, forming part of the boundary of an incisura interlobaris, with its afferent and efferent lymphatics is described. The focus from which tubercle bacilli were conveyed to the lymph node and the course of the lymphatics through which they passed were identified. Only in that portion of the lymph node which the lymphatic conveying the bacilli supplied were tubercles developed. The other lymphatics that entered the node formed a wide-open bypath through the node. The toxins contained in this bypath and in the associated sinuses gave rise to an intense hyperplasia of the lymphoid tissue in the remainder of the node. The lymph flow, as shown by the presence of numerous valves, was toward the pleura. This indicates that the direction of lymph flow described for other portions of the lung holds true in this situation; namely, that from a narrow zone around the periphery of the lung the flow is toward the pleura, while in other portions of the lung it is toward the hilum. Beyond the lymph node, the flow was through the pleural network of lymphatics to a hilum node, which showed active lesions. Lymphatics from other parts of the lung were seen entering the hilum node, showing that those described were not the only source from which it was infected.

H. J. CORPER.

TUBERCULOSIS OF THE COMMON CROW. CHARLES A. MITCHELL and R. C. DUTHIE, Am. Rev. Tuberc. **19**:134, 1929.

The incidence of tuberculosis in wild animals and birds in their natural state is largely a matter of conjecture and is governed in all probability by their relative chances of exposure rather than by any marked lack of susceptibility. In the case of *Corvidae* and birds of similar habits, contact with man and domesticated animals cannot be excluded. These birds might become infected in a number of

ways through contact with tuberculous poultry, domesticated animals and their dejecta, infected waste-products from garbage dumps and country slaughter-houses, and unburied carcasses of diseased animals. The susceptibility of *Corvidae* to mammalian tuberculosis is at present unknown. The ability of the crow to transmit infection to other birds or animals remains to be shown.

H. J. Corper.

Pregnancy and Pulmonary Tuberculosis. Arthur H. Morse, Am. Rev. Tuberc. **19**:140, 1929.

Attention is called to the most common theories supporting the contention that the reproductive process as a whole deleteriously influences a coexistent pulmonary tuberculosis. The relevant outstanding clinical observations appearing in the literature of France, Germany and the United States during the past decade are reviewed. Certain factors are considered which may explain the reason for the conflicting conclusions regarding the influence of pregnancy on pulmonary tuberculosis, and certain suggestions are offered as bearing on future studies of the problem.

H. J. Corper.

Multiple Pulmonary Abscesses from Blood Infection by the Proteus Group. M. J. King and R. H. Morgan, Am. Rev. Tuberc. **19**:182, 1929.

Recorded cases of human infection with *B. proteus* are infrequent. A case is presented in which positive blood cultures were obtained two hours after death. The micro-organisms were also cultured from lung abscesses and from pus in the frontal and maxillary sinuses. Repeated cultures of *B. proteus* were obtained from the sputum for several months before death. The organism was pathogenic for rabbits and guinea-pigs, and necrosis of the liver and heart muscles was produced in the experimental animals. A thermolabile toxin was produced in young broth cultures, which experimentally produced lesions comparable with those resulting from living micro-organisms.

H. J. Corper.

Hemoptysis in Tuberculosis Followed by Massive Pulmonary Atelectasis. Julius L. Wilson, Am. Rev. Tuberc. **19**:310, 1929.

Hemoptysis complicated by pulmonary atelectasis is extremely rare. The atelectasis may be logically attributed to the occlusion of a bronchus by a blood clot, but in no instance, apparently, has this been verified by recovery of the clot. The author reports a case in which a woman, aged 23, expectorated a bronchial cast composed of a blood clot. With this expectoration, the pulmonary atelectasis rapidly disappeared.

H. J. Corper.

Some Sources of Acid-Fast Bacilli. Stephen J. Maher, Am. Rev. Tuberc. **19**:376, 1929.

Acid-fast bacilli are forms of ordinary bacteria in which waxy capsules have developed to resist an unfavorable environment or forms resulting from the stimulation of acid-fast spores in an environment unfavorable to the full or ordinary evolution of these spores. Old tubercle bacilli, when suddenly transferred from an atmosphere saturated with their own ethers to one to which there is access of air, often grow wholly or partly free from their waxy capsules, and as rapidly as so-called pseudo tubercle bacilli. Acid-fast bacilli are to be found in, or can be cultivated from, several kinds of cancer. In internal cancer, they resemble the acid-fast colon bacilli, in uterine cancer the acid-fast bacilli and in cutaneous cancer either these same smegma bacilli or the coccal and ovoid acid-fast forms derivable from spore-bearing bacilli.

H. J. Corper.

TUBERCULOSIS AMONG THE AMERICAN INDIANS. STEPHEN J. MAHER, Am. Rev. Tuberc. **19**:407, 1929.

The American Indians, even in the wild, suffered from tuberculosis, and the doctrine that tuberculosis is a curse that the white race has passed along to the other races of the world is false.

H. J. CORPER.

THE SICKLE-CELL PHENOMENON IN TUBERCULOSIS PATIENTS. VERA B. DOLGOPOL and RICHARD H. STITT, Am. Rev. Tuberc. **19**:454, 1929.

The incidence of the sickle-cell phenomenon in seventy-seven tuberculous negro patients was found to be 5.2 per cent. The incidence of the sickle-cell phenomenon in 1,685 patients examined by different investigators was 6.5 per cent. With hereditary meniscocytosis, a greater prevalence of bizarre cells in the central portion of moist-chamber blood preparations is due to a greater asphyxiation of the erythrocytes in the center of the drop. Chronic anoxemia, caused by a disease or compression of the lung, is not an etiologic factor in the development of the meniscocytic anemia from hereditary meniscocytosis, nor can tuberculosis, syphilis and secondary anemia be regarded as etiologic factors.

H. J. CORPER.

THE TREND OF TUBERCULOSIS MORTALITY IN RURAL AND URBAN AREAS. EDGAR SYDENSTRICKER, Am. Rev. Tuberc. **19**:461, 1929.

The trend of the rural tuberculosis death rate was essentially identical with the urban tuberculosis death rate during the period from 1900 to 1915. Although the urban rate maintained itself with reference to the rural rate at a ratio of about 2 to 1, the proportionate decline was approximately the same in both populations. Immediately subsequent to the depression of the years 1914 and 1915 and in the influenza epidemic of 1918, a rise in mortality attributed to tuberculosis occurred; this was followed by a sharp drop in the rate of deaths from the disease in the years from 1919 to 1921. In these three years, the urban rate declined more rapidly than the rural rate. Since then, both rates have been declining somewhat more rapidly than in the period from 1900 to 1916. Without more detailed statistics, the following is offered: The net effect of the interplay of various factors on the tuberculosis death rate during the period from 1900 until the world war was approximately the same in urban and rural situations, but since then the forces favoring a decline have been somewhat more powerful in cities than in country districts.

H. J. CORPER.

EXPERIMENTS ON THE FILTRABILITY OF THE GRANULAR PHASE OF THE TUBERCLE BACILLUS. RALPH R. MELLON and ELIZABETH L. JOST, Am. Rev. Tuberc. **19**:483, 1929.

On injection of filtrates of tuberculous material from sixteen different sources, two animals of a total of thirty-seven yielded definitely positive results. Classic tuberculosis was reproduced, and the typical Koch bacillus recovered in pure culture. These positive results came only when the original material had been richly seeded with the granular form of the tubercle bacillus. Lesions which were possibly tuberculous also developed in two other guinea-pigs inoculated with filtrates from the same granular sources; these were not significant enough in the gross, however, to warrant further injection or culture. Evidence for the correctness of the filtration point of view can be based, in the beginning, at least, only on definitely positive results. In the absence of an isolatable tubercle bacillus from lesions that are histologically indistinguishable from known tuberculosis, one is not, with present knowledge of the life history of the tubercle bacillus, justified in drawing conclusions as to filtrability. Nor, on the other hand, is histologic evidence of tubercles in guinea-pigs of itself sufficient proof of filtrability. Such lesions have been caused by gram-negative paratyphoid-like micro-organisms. This was shown, not only by the isolation of the latter and

by their pathogenicity, but also by the failure of the histologically true tubercle to produce true tuberculosis when reinjected. The relation of this group of microorganisms to lesions indistinguishable from the necrobiotic type of tubercle lesions in guinea-pigs must always be kept in mind.

H. J. CORPER.

A STUDY OF PULMONARY TUBERCULOSIS IN CHILDREN. A. LEVINSON, Am. Rev. Tuberc. **19**:499, 1929.

In ninety-nine of 119 cases of active tuberculosis, mainly in negro children, pulmonary involvement was found. Sixty-nine were cases of miliary tuberculosis; fifteen, acute tuberculous pneumonia, and fifteen, subacute or chronic pulmonary tuberculosis. The reactions to tuberculin were negative in all cases of miliary tuberculosis. In some cases, the tuberculin reactions, which were positive when the patient entered the hospital, became negative when repeated after the onset of a miliary process. The pulmonary involvement in both the acute and the subacute forms was rather extensive and was mainly caseo-ulcerative. The prognosis in all the acute cases was bad. Of the fifteen patients in whom the disease was chronic, eleven had died, one was living, but the disease was running a progressive course, two were lost track of and one was doing well.

H. J. CORPER.

THE RELATIONSHIP BETWEEN BLOOD SEDIMENTATION INDEX AND FIBRIN CONTENT IN TUBERCULOUS PATIENTS. ESTHER M. GREISHEIMER, CHARLOTTE C. VAN WINKLE and OLGA H. JOHNSON, Am. Rev. Tuberc. **19**:559, 1929.

A significant relationship was found between the fibrin content and the sedimentation index in the groups studied. In both sexes, a significant correlation existed between the fibrin content and the amount of sedimentation, as determined at intervals of from fifteen minutes to one hour.

H. J. CORPER.

THIRTY STRAINS OF GRAM-POSITIVE COCCI ISOLATED FROM GENITO-URINARY INFECTIONS. ASYA M. S. STADNICHENKO, J. Bact. **17**:303, 1929.

Thirty strains of gram-positive cocci isolated from thirty cases of genito-urinary infection were studied diagnostically. Following are the principal results: The most prevalent type of infection in this series was prostatitis. However, no correlation was established between this type of infection and the strain of cocci isolated in connection with it. Strains in this series had a tendency to ferment carbohydrates strongly. The following carbohydrates were fermented by almost all the strains: dextrose, sucrose, maltose, mannitol and glycerol. The ability of strains in this series to decompose urea was pronounced. On the whole, the white strains exhibited more variation in their cultural characteristics than the orange. The orange group proved to have a higher percentage of gelatin-liquefying, nitrate-reducing, milk-coagulating and urea-decomposing strains. In general, the orange strains were more active, and the infections in connection with which they were isolated were more severe.

AUTHOR'S SUMMARY.

THE EFFECT OF FILTRATES OF CERTAIN INTESTINAL MICROBES UPON BACTERIAL GROWTH. MARGARET F. UPTON, J. Bact. **17**:315, 1929.

Bacillus coli and *Micrococcus ovalis* are inhibited in bacterial filtrates of cultures of *B. coli*, *M. ovalis* and *B. bifidus* at a p_H value of 4.5 and 5. The inhibition appears to be due to some factor in addition to that caused by an increase in hydrogen ions. Acetic and formic acids show an effect similar to that shown by filtrates, which disappears as the acids tend to become neutralized. Lactic acid has a less inhibitory effect than the other acids studied. Under the same conditions, *B. coli*, *M. ovalis* and *B. bifidus* utilize lactose differently, giving different proportions of volatile and lactic acids. The question is discussed as to the connection between the presence of large amounts of volatile acids presumably produced by *B. bifidus* in the

intestine of the breast-fed infant and the inhibition of *M. ovalis* and *B. coli* in that locality. The prevalence of these last two organisms in the feces of the artificially fed infant, in which the reaction is less acid, would seem to correlate with the results obtained from experiments outlined in this paper.

AUTHOR'S SUMMARY.

RELATIONSHIPS OF THE ENCAPSULATED BACILLI WITH REFERENCE TO BACT. AEROGENES. PHILIP R. EDWARDS, J. Bact. **17**:339, 1929.

The organisms that I have received from various sources labelled Friedländer bacilli cannot be distinguished from *Bacterium aerogenes* and the other members of the encapsulated group by action on milk or fermentative characters. Five cultures of *Bact. aerogenes* isolated from soil, water and milk have been found to be culturally, biochemically and serologically identical with type B of the Friedländer bacillus as described by Julianelle. Two cultures of *Bact. aerogenes* have been found serologically identical with a strain of the granuloma bacillus. *Bact. aerogenes* is so closely related to the other encapsulated forms that they should be classified in the same genus. No constant differences have been observed that could be used to separate the organisms into two or more species.

AUTHOR'S SUMMARY.

DIFFERENTIATION OF L. ACIDOPHILUS FROM L. BULGARICUS. WALTER L. KULP, J. Bact. **17**:355, 1929.

The results of these experiments indicate that strains of typical *Lactobacillus acidophilus* can be separated from strains of representative *Lactobacillus bulgaricus* by the determination of their tolerance for indol or phenol. The slight tolerance of *L. bulgaricus* for indol and phenol in experiments in vitro may help to explain why *L. bulgaricus* is not able to survive passage through the digestive tract. Further studies are planned to determine the validity of this theory.

AUTHOR'S SUMMARY.

OXIDATION-REDUCTION EQUILIBRIA IN BIOLOGIC SYSTEMS (POTENTIALS OF AEROBIC CULTURES OF B. TYPHOSUS). CALVIN B. COULTER and MOSES L. ISAACS, J. Exper. Med. **49**:711, 1929.

The reduction potentials of *Bacillus typhosus* in culture in bouillon that is given access at atmospheric oxygen show a negative drift which attains the values found in sterile bouillon deaerated with nitrogen: Eh —0.085 to —0.095 volt at p_H 7.6. The potential reaches this level after from six to eight hours' incubation and is maintained at this point for several hours. A slow decline to more negative values is then observed and continues for at least forty-eight hours, at which time a potential of —0.145 volt may be attained. The bacteria influence the potentials in the first period of their growth by exhaustion of oxygen from the culture, thus permitting the characteristic potential of the culture medium to become manifest, and do not contribute the substances responsible for the observed potentials. The decline in potential to values more negative than those of the culture medium occurs during the time that the rate of dying of the bacteria approaches and exceeds the rate of multiplication; it is suggested that dissolution of bacteria liberates reductive substances. Cultures in 0.5 per cent dextrose medium show a somewhat more negative potential after eighteen hours' growth than cultures in medium without dextrose. This may be due to the more rapid "turn-over" of the bacteria and the liberation of larger amounts of reductive material from the dissolution of larger numbers of bacteria. The potential of cultures through which oxygen is passed continuously does not show a negative drift at any time. This indicates that reductive substances of bacterial origin in the case, at least, of the typhoid bacillus do not influence the electrode potentials in the presence of oxygen and confirms the importance of bacterial respiration as the means for the removal of oxygen and the consequent establishment of characteristic reduction potentials in cultures.

AUTHORS' SUMMARY.

RELATION OF VARICELLA TO HERPES ZOSTER. T. M. RIVERS and L. A. ELDRIDGE, JR., J. Exper. Med. **49**:899 and 907, 1929.

Varicella most frequently occurs in persons under 10 years of age, while zoster as a rule is observed in persons beyond that age. The number of cases of varicella exhibits a markedly constant seasonal variation. The variations in the prevalence of herpes zoster are not regular and do not parallel those of varicella. Experiments and clinical observations dealing with the identity of the viruses of varicella and herpes zoster are presented. The results indicate that the etiologic agents concerned with these two diseases are in the majority of instances not identical.

AUTHORS' SUMMARY.

ETIOLOGY OF OROYA FEVER. THE INSECT VECTORS OF CARRION'S DISEASE. HIDEYO NOGUCHI, RAYMOND C. SHANNON, EVELYN B. TILDEN and JOSEPH R. TYLER, J. Exper. Med. **49**:993, 1929.

The experimental observations described in this paper lead us to conclude that certain phlebotomi act as vectors of Oroya fever and verruca peruana. The phlebotomi that have been shown certainly to carry *Bartonella bacilliformis* are those of the species *Phlebotomus noguchii. Phlebotomus verrucorum* is also probably a vector, while *Phlebotomus peruensis* in this respect remains doubtful.

AUTHORS' SUMMARY.

SOME NEW ASPECTS OF THE ETIOLOGY AND ENDEMIOLOGY OF LEPROSY. ERNEST LINWOOD WALKER, J. Prev. Med. **3**:167, 1929.

The confusion and doubt surrounding the cultivation of the lepra bacterium are due largely to the pleomorphic and facultative acid-fast character of this organism. The acid-sensitive or partly acid-fast coccoid, diphtheroid and actinomycoid organisms that have been cultivated repeatedly from leprosy are different stages in its life cycle. Hansen's bacterium in leprous lesions is probably the tissue stage. The organism belongs in the genus *Actinomyces,* as now constituted, and is most nearly related to the facultative acid-fast species, such as *Actinomyces asteroides* and *Actinomyces caprae.* The difficulty of cultivation of the lepra bacterium, the failure of experimental infections of man with leprous material and many of the clinical and pathologic peculiarities of the disease appear to be due, in part, at least, to the fact that the majority of the lepra organisms in the tissues of lepers are dead. Proof of the identity of the actinomyces cultivable from leprosy and Hansen's bacterium, like proof of the etiologic relation of the latter to leprosy, depends on the experimental reproduction of the disease in animals. Notwithstanding the absence of such proof, the evidence in support of both relations is convincing.

The actinomyces of leprosy, like other pathogenic actinomycetes, is a soil organism probably of wide but irregular distribution, and is only a facultative parasite. Leprosy probably is primarily an infection from the soil, presumably through wounds; but contagion as a possible secondary mode of dissemination is not thereby excluded. The bearing of these conclusions on methods of control of leprosy is obvious. It may ultimately be found that protection from soil-infected wounds and proper cleansing and disinfection of contracted wounds are at least as important as the segregation and isolation of lepers.

AUTHOR'S SUMMARY.

BACTERIAL STUDY OF HEMOLYTIC STREPTOCOCCI FROM A MASSACHUSETTS OUTBREAK OF SEPTIC SORE THROAT IN 1928. ELLIOTT S. ROBINSON and EDITH A. BECKLER, J. Prev. Med. **3**:225, 1929.

From the bacteriologic study of the outbreak of septic sore throat in Lee, Mass., in July, 1928, it appears that the causative organism was *Streptococcus epidemicus,* the same etiologic agent found by others in previous outbreaks. This organism was found in the throats of a number of patients, in the aural discharge of some and in the blood of one. The milk-borne nature of the epidemic is shown by the

epidemiologic evidence presented by Lombard and by the finding of *S. epidemicus* in a cow that had been providing part of the milk consumed by the patients. The source of the cow's infection remains undetermined and probably undeterminable. By the usual criteria, *S. epidemicus* is of human, not bovine, origin, and it is reasonable, therefore, to suspect a human source. Although there was illness on the farm at a time when the cow might be supposed to have been infected, the epidemiologic evidence is not of itself sufficient to connect this illness with the infection of the cow, and there is not at present bacteriologic evidence to link the two. The isolation of *S. epidemicus* from the throats of patients is not difficult if one is familiar with the colony typical of this organism. Since there may be little hemolysis around the surface colony when grown on a moist medium, it may be overlooked; for the colony does not greatly resemble that of the more usual hemolytic streptococci. Culturally, the strains of *S. epidemicus* obtained during this epidemic appear identical with those isolated during previous outbreaks.

AUTHORS' SUMMARY.

NEGATIVE RESULTS OBTAINED IN THE ATTEMPT TO RELATE TUBERCULOSIS SUSCEPTIBILITY OF RESISTANCE TO A PARTICULAR BLOOD GROUP. LELAND W. PARR, J. Prev. Med. **3**:237, 1929.

The extensive literature dealing with the possible correlation of some particular blood group and susceptibility or resistance to tuberculosis is contradictory. The positive evidence indicates susceptibility in group A and resistance in group O. Entirely negative results are more numerous, however, and apparently more significant. The data here presented from 346 cases of clinical tuberculosis in Syria, in which tests for blood groups were made, fail to support the contention that there is any relation between susceptibility to tuberculosis and any one blood group. Similarly, no resistance to tuberculosis could be demonstrated for any one of the blood groups. In a series of tuberculin and blood-grouping tests, the 450 persons whose reactions to tuberculin were positive fell within the blood groups in the same proportions as the entire series of 944 persons. We conclude that all measures for the prevention and cure of tuberculosis should be applied to persons equally, irrespective of their blood groups, as persons of no one blood group appear to be significantly susceptible or particularly resistant to tuberculosis.

AUTHOR'S SUMMARY.

A STUDY OF THE SIGNIFICANCE OF GEOGRAPHIC AND SEASONAL VARIATIONS IN THE INCIDENCE OF POLIOMYELITIS. W. LLOYD AYCOCK, J. Prev. Med. **3**: 245, 1929.

There is evidence that, in man, infection with the virus of poliomyelitis manifests itself in different ways: frank disease in relatively few and immunization without recognizable disease in the majority. Data presented in this paper indicate that these different reactions to infection with the virus may be due to variations in the physiology of the host, rather than to variations in the virus. Intracerebral inoculation of the virus in monkeys uniformly produces fatal poliomyelitis, and repeated intracutaneous inoculation of active virus produces immunity without symptoms of the disease, but neither of these methods reproduces an attack of the same degree of severity as that seen in man. It was found, however, that intranasal instillation of the virus reproduces poliomyelitis of nearly the same order of severity as that observed in man. The disease developed in only a portion of the animals following intranasal installation of the virus, and—what seemed to be more significant—in many of the animals a much milder form developed than that which practically always follows intracerebral inoculation. Furthermore, in one instance in which the animal showed no symptoms following intranasal inoculation, its blood serum in three separate tests was found to be capable of neutralizing the virus. From this, it appears that monkeys may possess a resistance to intranasal instillation of the virus not unlike that which man possesses to infection by natural means. It

may be possible, therefore, to gain further knowledge of this form of resistance to poliomyelitis (autarcesis) by testing the influence of artificial alterations in physiologic functions of the monkey on the results of intranasal instillation of the virus.

AUTHOR'S SUMMARY.

TUBERCULOUS ORIGIN OF IRIDOCYCLITIS. A. V. FRISCH and A. PILLAT, Arch. f. Ophth. **121**:504, 1929.

The authors believe that tubercle bacilli can produce every form of iridocyclitis from the simplest serous type to a panophthalmitis. The intracutaneous test for tuberculosis is the most sensitive and is harmless. On the basis of a positive intracutaneous tuberculin reaction and the general condition, together with an increased rate of sedimentation of the red corpuscles, which occurs frequently, a large number of cases of chronic recurring iridocyclitis can be considered as due to tubercle infection, in spite of the absence of typical tubercles. A large number of the cases of what was previously named "rheumatic" iridocyclitis belong to this group. The rate of sedimentation of the red blood corpuscles is increased in about 50 per cent of the cases of nodular iritis. The cases with a normal rate of sedimentation appear to proceed more favorably.

CHARLES WEISS.

PERISPLENITIS AND THE DISCOVERY OF SPIROCHETES IN CONGENITAL SYPHILIS. J. WATJEN and J. MUNZESHEIMER, Virchows Arch. f. path. Anat. **269**:325, 1928.

New and old perisplenitis occurs in more than one half of the cases of congenital syphilis and is often found accompanied by splenomegaly. This perisplenitis may be specific, for spirochetes were found in the splenic capsule and the perisplenic deposits. Spirochetes may wander into the capsule from the tissue immediately underneath it; also by way of the trabeculae, so that their deposition corresponds to the course of the connective tissue strands. Spirochetes may reach the peritoneal cavity from the splenic capsule, as demonstrated in six cases. That the reverse occurs, namely, perisplenitis from spirochetes within the peritoneal cavity, is not so probable.

STANLEY P. REIMANN.

REACTION OF THE VESSELS IN THE ISOLATED RABBIT'S EAR TO STREPTOCOCCI. A. P. ANOCHINA-IWANOWA, Ztschr. f. d. ges. exper. Med. **63**:792, 1928.

The author has previously reported experiments with streptococci and their toxins on vessels of the rabbit's ear isolated after the method of N. Krawkow. It was found that streptococcic (scarlet fever) toxin leads to a narrowing of the vessels in the isolated ear and later to edema of the tissue. These reactions are most pronounced if the temperature of transfusing fluid is from 37 to 39 C.

BALDUIN LUCKE.

LIBERATION OF TOXINS AND HEMOTOXINS FROM BACTERIA SUSPENDED IN BROTH AND IN SOLUTIONS OF DIFFERENT SALTS. N. FUJIOKA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **57**:466, 1928.

The experiments were made with the hemotoxin of cholera vibrios and the toxins of dysentery and of diphtheria bacilli. Suspensions of the bacteria were made in broth and in solutions of different salts, and the liberation of the toxin or hemotoxin was determined after short periods of extraction.

The bacterial suspensions in NaCl and KCl solutions liberated more toxin than similar suspensions in $CaCl_2$, $BrCl_2$ or $MgCl_2$. Diphtheria bacteria liberated more toxin in a two hour extraction period if suspended in NaCl solution than if suspended in broth; after longer periods, more toxin was liberated in the broth, owing to growth of the bacteria in the culture medium.

The author believes that the liberation of the toxin or hemotoxin depends on the influence of the different salts on the permeability of the bacterial cell.

R. C. AVERY.

LYMPHOGRANULOMA INGUINALE. SVEN HELLERSTRÖM, Acta derm.-venereol., 1929, suppl. 1.

It appears that inguinal lymphogranuloma can be traced back to the middle of the eighteenth century, and probably existed earlier. In Sweden, it was first described as a specific disease in 1927, but it is likely that it was observed by Ödmansson in 1887, and intracutaneous reactions indicate that it has existed there since about 1903.

A detailed account is given of forty-seven cases, two of them in women. In twenty-five cases, alterations were seen on the genitalia, the greater number being interpreted as primary. They consisted of erosion, superficial ulcer, nodule or papule, and urethritis; sometimes mixed chancre may have been concerned. The incubation from the probably infecting coitus to the appearance of adenitis is about one month. One "partner-case" as it is called, with isolated coitus, is described. In this, the periods of incubation were sixteen and twenty-one days. The city of infection in thirty-two cases was stated to have been Stockholm; in the remainder of the cases, the infection was acquired abroad. Erythema nodosum was observed in four cases, and inguinal lymphogranuloma is to be included among the infectious diseases which may be accompanied by erythema nodosum.

In every one of the forty-seven cases there was an undoubtedly positive reaction to Frei's intracutaneous test (extract of the pus in the affected lymph glands) for inguinal lymphogranuloma, carried out with seven different antigens, one of which had been obtained from Frei in Breslau. The antigens were tested and compared with each other, auto-antigen alone not being employed. In more than 60 per cent of the cases, Frei's reaction was carried out within a period of from one to three weeks to two months after the adenitis had been observed. The reaction was always positive. The persistence of cutaneous allergy, in one instance, was for twenty-four and a half years. It has not been possible to obtain any allergic reaction in healthy persons by intracutaneous injections of the antigen. If proper precautions are taken, the antigen remains active for more than a year. The Frei intracutaneous reaction appears to be specific.

Pathologic examinations and attempts to transfer the disease to animals resulted negatively. The anatomic picture in inguinal lymphogranuloma is not pathognomonic, and is, therefore, not sufficient for a diagnosis. Not all buboes are due to inguinal lymphogranuloma. In cases of inguinal adenitis, the etiology must be established by every possible means, before steps are taken to determine the histologic picture. Among the negative control-cases of Frei's reaction, were seventeen of ulcus molle, twelve with buboes.

The crossed antigen-tests between inguinal lymphogranuloma and climatic bubo, which resulted positively and which were verified by Fischer and Frei, appear to confirm the theory that inguinal lymphogranuloma and climatic buboes are identical. It has been impossible to determine any clinical or anatomic difference between the cases in which infections occurred in the tropics (climatic bubo) and those in which it occurred in Sweden.

There is described one case of extragenital localization of inguinal lymphogranuloma inguinale with positive intracutaneous reaction, which supports the theory that inguinal lymphogranuloma is a disease *sui generis*.

AUTHOR'S SUMMARY.

Immunology

IMMUNIZATION AGAINST DIPHTHERIA WITH RAMON'S TOXOID. GEORGE F. DICK and GLADYS H. DICK, J. A. M. A. **92**:1901, 1929.

Ramon's toxoid was found a better immunizing agent than toxin-antitoxin.

BLOOD CALCIUM DISTRIBUTION IN ANAPHYLAXIS IN THE GUINEA-PIG. HERMAN BROWN and SUSAN GRIFFITH RAMSDELL, J. Exper. Med. **49**:705, 1929.

The results reported for total calcium and the membrane-diffusible fraction in the serum of the guinea-pig, taken at various intervals during anaphylactic shock,

confirm the observations of previous workers that the total calcium is essentially unchanged. There is, however, the further observation that the diffusible fraction is considerably increased over that found for the animal similarly treated but not manifesting characteristic symptoms.

AUTHORS' SUMMARY.

TRANSMISSION OF RESPIRATORY ANAPHYLAXIS (ASTHMA) FROM MOTHER TO OFFSPRING. BRET RATNER and HELEN LEE GRUEHL, J. Exper. Med. **49**: 833, 1929.

A further method is offered whereby sensitization in utero may be established. Respiratory anaphylaxis—induced in a pregnant guinea-pig by the inhalation of a dry antigenic dust—can thus be transmitted from mother to offspring. A guinea-pig thus sensitized in utero, when brought into contact for the first time with an anaphylactogenic dust to which the mother was sensitized, will manifest respiratory anaphylaxis. The transmission of this hypersensitiveness may be brought about passively through the transmission of sensitizing antibodies. A fetus may be actively sensitized in utero by a mother who has inhaled the antigenic dust and has not herself been sensitive at the time of birth. This state of hypersensitiveness may be transmitted in varying degrees of intensity, and when two or more offspring are born in the same litter, they may, in some instances, be sensitized to an equal degree and sometimes to different degrees. This state of hypersensitiveness can be transmitted through more than one litter. All animals cannot be made hypersensitive.

AUTHORS' SUMMARY.

A "SOLUBLE SPECIFIC SUBSTANCE" DERIVED FROM GUM ARABIC. MICHAEL HEIDELBERGER, OSWALD T. AVERY and WALTHER F. GOEBEL, J. Exper. Med. **49**:847, 1929.

By partial acid hydrolysis a specific carbohydrate may be isolated from gum arabic (acacia). This carbohydrate is comparable in its precipitating activity for type II and (type III) antipneumococcus serum with the bacterial soluble specific substances themselves. On hydrolysis this fraction yields galactose and two or more complex sugar acids, one of which appears to be a disaccharide acid comparable with those isolated from the specific polysaccharides of the type III pneumococcus and the type A Friedländer bacillus. The significance of these observations is discussed.

AUTHORS' SUMMARY.

SKIN REACTIONS IN RABBITS IMMUNIZED INTRAVENOUSLY WITH NON-HEMOLYTIC STREPTOCOCCIC. HOMER F. SWIFT and C. L. DERICK, J. Exper. Med. **49**:883, 1929.

Rabbits immunized intravenously with living culture or nucleoproteins of nonhemolytic streptococci react to subsequent intracutaneous inoculations with homologous streptococci with smaller and harder lesions than are shown by normal animals similarly inoculated; they do not show the general manifestations of hypersensitiveness such as are shown by animals the tissues of which were previously inoculated with the same cultures. A rabbit may react to intracutaneous inoculation with nonhemolytic streptococci in one of four ways, depending on whether it is normal, hypersensitive, immune or cachectic. Most normal animals show a secondary reaction about ten days after inoculation with suitable strains of nonhemolytic streptococci; hypersensitive, allergic, or hyperergic animals show much larger lesions than do normal ones with the corresponding doses of the same streptococci, and practically never show secondary reactions; immune animals show smaller and harder early lesions and usually do not have secondary reactions if they are fairly well immunized. Cachectic animals show soft and rapidly fading primary reactions and no secondary reactions.

AUTHORS' SUMMARY.

IMMUNOLOGIC REACTIONS WITH TOBACCO MOSAIC VIRUS. HELEN A. PURDY, J. Exper. Med. **49**:919, 1929.

Evidence is presented that a specific antibody to virus sap, lytic in nature, is present in the homologous antiserum from rabbits into which sap from tobacco plants affected with mosaic disease has been injected.

INTRADERMAL VERSUS SUBCUTANEOUS IMMUNIZATION OF MONKEYS AGAINST POLIOMYELITIS. F. W. STEWART and C. P. RHOADS, J. Exper. Med. **49**: 959, 1929.

The introduction of considerable amounts of living, active poliomyelitis virus into the skin and subcutaneous tissue of monkeys protects the animals against intracerebral inoculations of similar virus material. The degree of protection conferred by intradermal is greater than that by subcutaneous injection. During intradermal and subcutaneous inoculations, no local or general pathologic signs were observed. The degree of protection produced by the immunization methods used is not absolute, since a percentage of the inoculated monkeys respond to intracerebral injections of highly potent virus. The serums of the animals inoculated intradermally or subcutaneously neutralized poliomyelitis virus in vitro, irrespective of the result of intracerebral inoculation, in all except one instance. The power of the serum of treated monkeys to neutralize virus in vitro is a more delicate test of immunity than is the intracerebral inoculation.

AUTHORS' SUMMARY.

YELLOW FEVER VIRUS. N. C. DAVIS and A. W. BURKE, J. Exper. Med. **49**:975 and 985, 1929.

While there are quantitative differences in virulence and minor differences in behavior, the African strain and the Brazilian strains of yellow fever virus studied in this work are immunologically the same.

ACQUIRED IMMUNITY IN AVIAN MALARIA: IMMUNITY TO SUPERINFECTION. WILLIAM H. TALIAFERRO and LUCY GRAVES TALIAFERRO, J. Prev. Med. **3**: 197, 1929.

Immunity to superinfection in bird malaria is evidenced by the fact that when large numbers of washed parasitized blood cells are introduced into the blood stream of a bird during the latent infection, they are quickly removed, whereas in normal birds they not only live, but increase rapidly in numbers. A quantitative study of the degree of immunity to superinfection—that is, the number of parasites that are removed, and the rate of removal by the bird during the latent infection—indicates that when the number of parasites injected into latent birds is approximately from 1 to 100 parasites per ten thousand red cells, the parasites are removed from the peripheral blood wthin twenty-four hours. If the number of parasites is increased from 100 to 400 per ten thousand red cells, they are removed within from forty-eight to seventy-six hours. The degree of immunity to superinfection may be less at the beginning of the latent infection than later. Removal of the parasites of birds during the latent infection takes place at all stages of the asexual cycle. It cannot be considered a phagocytosis of only the merozoites which are free in the serum. A high degree of immunity to superinfection was found as long as six hundred and fifty-six days after the primary inoculation of infected organisms.

AUTHORS' SUMMARY.

ACQUIRED IMMUNITY IN AVIAN MALARIA. THE ABSENCE OF PROTECTIVE ANTIBODIES IN IMMUNITY TO SUPERINFECTION. WILLIAM H. TALIAFERRO and LUCY GRAVES TALIAFERRO, J. Prev. Med. **3**:209, 1929.

The first report of this series demonstrated a highly effective parasiticidal mechanism in birds with a latent infection (immunity to superinfection). The present report demonstrates that this mechanism is not associated with a humoral antibody.

STREPTOCOCCUS TOXINS. H. VON HIRSZFELD, M. MAYZNER and F. PRZESMYCKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **57**:414, 1928.

Forty strains of streptococci from scarlet fever, erysipelas and other sources were tested for toxins reactive with human skin. Positive results were obtained with almost all strains. Immunologic differences between the toxins were found in cross neutralization experiments with scarlet fever and erysipelas toxins and convalescent serums. In most cases the scarlet fever convalescent serums neutralized the toxin of scarlet fever strains but not the toxin of erysipelas strains. The erysipelas convalescent serums were less specific and usually neutralized the toxin of scarlet fever as well as the toxin of erysipelas strains.

R. C. AVERY.

INFLUENCE OF INJECTIONS OF SPLEEN EXTRACT ON IMMUNITY RESPONSE OF GUINEA-PIGS. HANS SCHLACK, Ztschr. f. Immunitätsforsch. u. exper. Therap. **57**:499, 1928.

The paper reports that the parenteral injection of spleen extract (freed from albumin and lipoids) increases the immunity response of guinea-pigs to smallpox vaccine, diphtheria bacilli and staphylococci. Negative results were obtained in similar experiments with pneumococci. The author believes that the action of the spleen extract can best be explained as due to a stimulation of the reticular endothelial apparatus.

R. C. AVERY.

Tumors

THE PRODUCTION OF SARCOMAS BY THE IMPLANTATION OF EMBRYONIC PULP AND INACTIVATED CARCINOMATOUS PULP. VINCENZO BISCEGLIE, Tumori **3**:139, 1929.

No development of tumor was obtained by the simultaneous inoculation of rats with embryonal pulp and filtrates of carcinoma from rats. The pulp of carcinoma from rats is inactivated by exposure to chloroform (10 per cent) for three hours so completely that its inoculation remains constantly and completely negative. By simultaneous inoculation of inactivated pulp of carcinoma and embryonal pulp into twenty-five rats there was obtained in two animals a development of growths which on histologic examination proved to be sarcomas and which had given rise to metastases in the lungs and liver. One of these tumors was successfully transplanted. The sarcomatous tumors had probably taken their origin from embryonal connective tissue cells which had been exposed to the action of a tumor-producing agency in the inactivated carcinomatous pulp. The latter is probably a chemical product elaborated by cells the metabolism of which has been altered by various irritative agents.

W. OPHÜLS.

THE EPITHELIAL TUMORS OF THE BLADDER. ALFREDO FONTANA, Tumori **3**: 185, 1929.

Fontana has studied eighteen specimens, fourteen obtained at autopsy and four at operation. At the pathologico-anatomic institute at Milano, 12 epithelial tumors of the bladder were found among 2,000 autopsies. He distinguishes benign and malignant papillomas and believes that the majority of carcinomas, especially the papillary ones, are derived from them. He also encountered typical primary adenocarcinomas of the bladder, associated with adenomatous proliferation of the prostate. He believes that in such persons there is a special proliferative activity of the vesicoprostatic glandular epithelium and that the physicochemical stimulus which produces it causes the malignant degeneration first in the bladder. The rare cases of malignant epithelioma of the bladder arise from areas of leukoplakia.

Contrary to common belief, Fontana found metastases in other organs in several cases of carcinoma of the bladder, among them twice in the suprarenals and twice in the bones. He believes that these tumors of the bladder develop in especially

predisposed organs as a result of various physicochemical stimuli. Other predisposing factors are stones, exstrophy and diverticula.

W. OPHÜLS.

THE RELATION BETWEEN CARCINOMA AND LIPID METABOLISM. F. BURGHEIM, Klin. Wchnschr. **8**:828, 1929.

In contrast with other diseases, the cholesterol in the blood of patients with carcinoma is increased after roentgen treatment. This variation disappears when the tumor is removed surgically. Malignant tissues contain large amounts of cholesterol, benign tissues, little or none.

AUTHOR'S SUMMARY.

NEUROBLASTOMA OF THE SYMPATHETIC NERVE. C. BLUMENSAAT, Virchows Arch. f. path. Anat. **269**:431, 1928.

Exceptionally, neuroblastoma of the sympathetic nerve may occur in adults. Pure sympathogoniomas have not been described in adults.

STANLEY P. REIMANN.

MIXED TUMORS OF THE LIVER. WERNER NISSELL, Virchows Arch. f. path. Anat. **269**:446, 1928.

Mixed tumors of the liver are extremely rare. In the case reported, death resulted from hemorrhage due to rupture of the tumor. In addition to epithelial and connective tissue and parts histologically malignant, the tumor also contained cartilage and bone. The structure probably arose from epithelial and connective tissue elements in the liver anlage. The development of cartilage and bone may be explained by progressive metaplasia in Lubarsch's sense.

STANLEY P. REIMANN.

THE FREQUENCY OF PULMONARY CARCINOMA AND THE CAUSES OF ITS INCREASE. E. VON ZALKA, Ztschr. f. Krebsforsch. **26**:130, 1928.

A study of the relative incidence of primary carcinoma of the lung in autopsy statistics from the hospital from 1894 to 1927 showed an increase in that time from 1.02 to 6.65 per cent of all cases of cancer. The rise occurred principally during the years after 1923. As regards etiology, while following influenza there are frequently changes in the lung of precancerous type, the importance of this infection as an agent is discounted by the frequent absence of a history of previous influenza. A second possible factor is that of exposure to war gases, also associated with altered epithelial relationships. A third conjectural factor is that of dietary insufficiency, since deficiency of vitamin A in experimental animals has been found to cause epithelial metaplasia. Of the possible factor of irritation by exhaust gases from internal combustion engines the writer makes only passing mention.

H. E. EGGERS.

THE RÔLE OF THE BLOOD VESSELS IN THE GENESIS OF TAR TUMORS. L. KREYBERG, Ztschr. f. Krebsforsch. **26**:191, 1928.

The application of coal tar to the skin of white mice produces a marked local and lasting hyperemia, which appears to have a direct relationship to the ensuing epithelial hyperplasia. Effects which have been ascribed to the local alterations of nerve supply in these tumors may with equal warrant be ascribed to the vascular changes.

H. E. EGGERS.

THE NATURE AND THE CLINICAL VALUE OF THE CANCER REACTION OF ROFFO. H. HILAROWICZ and W. JANKOWSKA-HILAROWICZ, Ztschr. f. Krebsforsch. **26**:214, 1928.

A study of the Roffo reaction for cancer — consisting of the addition of aqueous solution of neutral red to the serum to be tested, with a red coloration indicating

the presence of malignant disease — led to the conclusion that the altered reaction was due to the presence of increased globulin content in the serum. Clinical application did not show it to be of any great diagnostic value.

H. E. Eggers.

Humoral and Cellular Growth Factors of Cancer Cells. A. Fischer, Ztschr. f. Krebsforsch. **26**:228, 1928.

Carcinoma cells are capable of obtaining nutritive material, as shown by continued and progressive growth in vitro, from serum and from inactivated embryonal extracts, differing in this respect from normal tissues. Also, carcinoma cells showed continued growth if they were in direct contact with fibroblasts, even if cultural conditions no longer permitted the growth of the latter.

H. E. Eggers.

The Measurement of Growth of Carcinoma Cells in Vitro. A. Fischer and H. Laser, Ztschr. f. Krebsforsch. **26**:235, 1928.

The residual energy and the inherent growth energy of carcinoma cells in Tyrode's solution were found to be somewhat less than those of normal tissues. If to the carcinomatous cells in vitro there were added normal tissues, the former showed an increased residual energy, which may be ascribed to the summation of the nutritive reserve of both tissues. Carcinoma cells grown in serum showed a considerable enhancement of growth if they were placed previously in contact with normal cells. By this contact they obtained an accessory principle, not nutritive in character, which acted as an accelerator of anabolism from the nutritive elements of the serum. This accessory factor is one of the important causes of unlimited proliferation in the organism. Carcinoma cells were found to be sensitive to embryonal extracts and to certain preparations of proteoses; with increased concentration of these there was an increase in the rate of growth, but with high concentrations the cells died rapidly.

H. E. Eggers.

The Growth of Carcinoma Cells and the Hydrogen Ion Concentration of the Medium. A. Fischer, Ztschr. f. Krebsforsch. **26**:250, 1928.

Carcinoma cells were found to be very sensitive to variations of the hydrogen ion concentration of the medium. At a p_H of 5.9 growth no longer continued, although fibroblasts continued to grow for several days. On the other hand, cancer cells were less affected by increased alkalinity. The optimal hydrogen ion concentration for growth of malignant tumor cells is at least not more acid than that of normal tissues.

H. E. Eggers.

The Practical Value of the Interferometric Methods in the Diagnosis of Cancer. F. P. Tinozzi, Ztschr. f. Krebsforsch. **26**:286, 1928.

The writer concludes that while the interferometric examination of blood serum offers so many complications, especially in the way of technical difficulties, it is of little practical value at present; improved and simplified methods may ultimately prove of importance in the diagnosis of malignancy.

H. E. Eggers.

The Influence of Vitamin Unbalance on the Origin of Malignant Tumors. R. Erdmann and E. Haagen, Ztschr. f. Krebsforsch. **26**:333, 1928.

In a small percentage of rats (three of eighty-three animals surviving over four weeks) fed on a diet deficient in all vitamins except B and D, with the latter given in excess, there occurred spontaneous tumor development, which was shown by none of the control animals of similar descent on normal diets. The writers admit the possibility of congenital sensitiveness to vitamin unbalance. The latter condition they assume results in a lowering of cell unions, especially those of the reticulo-

endothelial system, by which the appearance of cell aggregates in reaction to external stimuli is favored, and these aggregates ultimately develop into tumors.

H. E. Eggers.

Nuclear Measurements in Tar Cancers of White Mice. W. Epantschin, Ztschr. f. Krebsforsch. **26**:439, 1928.

Following the continued application of coal tar to the skin of the white mouse, there is an increase in the size of the epithelial nuclei. The nuclei of the cells of tar cancers are characterized by marked polymorphism and increased size, comparable in these respects to those of cancers of the skin in men. In corresponding cancroidal tissues the polymorphism and increased size are present, but to a lesser degree.

H. E. Eggers.

The Biology of Cancer Cells in Vitro. A. Fischer, Ztschr. f. Krebsforsch. **26**:463, 1928.

Fischer's views of the biology of cancerous tissues, as revealed by tissue cultures, may be summarized as follows: Cancer cells are distinguished, even outside the living organism, by certain properties which they transmit to their offspring. Their enhanced growth is achieved by their ability to utilize as foodstuffs materials unsuited for normal cells. Also, they obtain a growth-stimulating factor by contact with normal cells. The differences in dynamic metabolism and in reactions to injury are revealed by their behavior in vitro. All differences are probably to be viewed as of quantitative rather than of qualitative character.

H. E. Eggers.

Organ Changes in Mice After Application of Coal Tar. W. Berghoff, Ztschr. f. Krebsforsch. **26**:468, 1928.

Following the long continued application of coal tar to the skin of white mice, three types of tissue change occur. Many animals show marked degenerative changes in the liver and kidneys; there is cellular proliferation, apparently of the reticulo-endothelial system, in the liver and spleen, and amyloid infiltration was seen especially in the spleen, more rarely in the liver and kidney. That these changes, especially the amyloid change, are not to be ascribed entirely to tumor action is shown by the fact that they occurred in animals without tumor. In either case their inception is probably to be ascribed to disturbance of the protein metabolism. All these changes were more pronounced in animals with developed tumors, which the writer interprets as indicating that tumors are most apt to form in those animals showing greatest organic injury. On the other hand, a series of mice fed with a diet high in iodized oil developed a high percentage of tumors, without showing amyloid infiltration. Evidently, the factors producing amyloidosis are not identical with those producing tumor. In general, Berghoff regards the degenerative changes as an indication of metabolic disorder which predisposes the animal to tumor formation, with the external irritation acting as the direct cause.

H. E. Eggers.

Medicolegal Pathology

Resistance of Criminals Against Accidental and Surgical Traumatisms. C. Gasparini, Arch. di antrop. crim. **48**:1, 1928.

An interesting discussion is based on several clinical observations illustrating a peculiar, extraordinary resistance of criminals against various kinds of physical and psychic traumatisms, such as severe injuries, operative procedures, dangerous infections, etc., a fact which Lombroso already had emphasized and designated as "disvulnerabilità." It is a characteristic somatic behavior exhibited by real criminals, and forms an important subject in the study of biology of criminals.

Similar observations can be made also among certain types of prostitutes, since prostitution can be regarded as a degenerative equivalent of delinquency. Such prostitutes show many somatic and psychic stigmas of degeneration, such as hypoalgesia, intellectual deficiency, impudence, aversion to work, etc. In spite of all possible and conceivable privations, exposures and hardships, the criminal remains unaffected. And in this respect there is a striking difference between the real, common criminal and the so-called political criminal. The mortality of the first group is low compared with that observed in short-termed prisoners. This surprising physical resistance is particularly evident toward abdominal wounds of any kind, ingestion of foreign bodies, etc., and in rapidity of recovery, so that Martini called the peritoneum of a criminal a "dog's peritoneum." Gasparini concludes that the biologically peculiar resistance of criminals against accidental and surgical traumatisms forms one of the degenerative signs of delinqency.

E. L. MILOSLAVICH.

DISTRIBUTION OF THE BLOOD GROUPS AMONG THE CRIMINALS IN PIEMONT. G. CANUTO, Arch. di antrop. crim. **48**:687, 1928.

Among Piemontese criminals, an increase in the blood group B was observed with a corresponding decrease in groups A and AB; the A:B index is smaller than the racial biochemical index of the normal population of Piemont. The preponderance of group B was found in persons who repeatedly committed one and the same delinquent act, as well as among those who had already been sentenced several times. In an analogous way, group B was often met with in criminals who were sentenced for various crimes. But a decided increase of group B was found in instances of homicide, robbery, violence, rape and rebellion, particularly if the perpetrators of these crimes were contrasted with other types of deliquents. There is an unmistakable correlation between group B and the presence of the somatic characteristics and external signs of degeneration that constitute the criminal type.

E. L. MILOSLAVICH.

PRACTICAL VALUE OF CRYSTALLIZATION OF HEMOGLOBIN FOR DIAGNOSIS OF BLOOD. F. NICOLETTI, Arch. di antrop. crim. **48**:705, 1928.

Even prior to the time of the discovery of the precipitin test, crystallization of hemoglobin was intensively studied for medicolegal purposes in order to establish a reliable and specific method for differentiation of blood of various animal species. Falco was the first who studied it with the method of Amantea, using the highly hemolytic substance *Saponaria officinalis;* he believed that he was able to differentiate between human blood (adult as well as new-born) and that of other animals. The author now reports results of his investigations regarding human and animal blood, employing not only saponin, but also other chemicals, such as sodium fluoride and ammonia, particularly for fluid or clotted blood. From the results obtained, he concludes that only certain animals (guinea-pig, mouse, rat, squirrel) possess a constant and characteristic type of hemoglobin crystallization which allows one not only to diagnose the species in question, but also to differentiate between human blood and that of other animals. Some animals (dog, cat, hog, horse, rabbit, chicken, man, etc.), however, do not show any specific morphologic peculiarities that could be used to determine successfully the respective species. In some animals (guinea-pig, mouse, horse, rabbit), the blood pigment crystallizes only while in the state of oxyhemoglobin; in other species (cat, man, etc.) exclusively as reduced hemoglobin, while the blood pigment of such animals as dog, chicken and hog produces crystals from reduced hemoglobin, as well as from oxyhemoglobin. From all the methods described to date to obtain crystallization of hemoglobin, the best one is the saponin method of Amantea. Such a crystallization is also readily achieved if one uses tungstate, molybdate, phosphate and particularly sodium sulphate. The blood pigment of a given species generally needs for crystallization a definite period of time, which differs from that required by the blood pigment of another species (for instance, that of a guinea-pig or a

mouse will crystallize in from a few minutes to from one to four hours, that of a hog in from eighteen to twenty hours). This phenomenon is independent of any physical or chemical influences. The individual crystals, observed under a polarizing microscope, do not present any optic properties that would enable one positively to differentiate between the hemoglobin of various animal species. The following facts would, with great probability, permit the diagnosis of human blood: (1) the presence of crystals of reduced hemoglobin; (2) occurrence of crystals in exclusively tabular form (rectangular tables); (3) a time period for the crystallization of not less than twenty-four hours; (4) after a few days a tint of violet in the hemoglobin crystals (not noticeable in other species), and (5) a relatively short durability of the specimens prepared.

E. L. MILOSLAVICH.

MENTAL AND PHYSICAL COMPETENCY FOLLOWING A BULLET WOUND OF THE HEAD. W. WEIMANN, Arch. f. Kriminol. **82**:178, 1928.

A man noticed no ill effects after shooting himself in the right temple. He felt the bullet pass out of the left temple. He lay in bed, but was completely oriented, and at a judicial hearing the next day made a clear statement. The wound healed after brain tissue and particles of bone had escaped and trephining had been done for abscesses that developed. It was assumed that the anterior horns of the lateral ventricles had not been opened and that certain so-called "blind" regions of the brain were the only parts injured.

E. R. LECOUNT.

SUBENDOCARDIAL HEMORRHAGE AND ITS SIGNIFICANCE. J. GERINGER, Beitr. z. gerichtl. Med. **8**:105, 1928.

Subendocardial hemorrhages were found more frequently in bodies examined because the deaths were definitely subject to medicolegal inquiry than in those examined simply because death was not expected and health regulations in Vienna required their examination. In the first group there were only twenty bodies with subendocardial hemorrhages in 1,740 (1.2 per cent) examined post mortem; in the second group, the hemorrhages were in eighty-two of 707 bodies (11.5 per cent). Their cause, location, size and relation to the bundle of His conduction bands are reviewed with the literature and many tables of statistics. It was found that they are more frequent with death from hemorrhage and especially internal hemorrhage. Injuries of the head, particularly when there is an accompanying compression of the brain, metallic poisons, convulsions, apoplexy and tumors of the brain are other important causes. The hemorrhages as a rule are under the lining of the septal portion of the left ventricle; in only three hearts were they in the right ventricle.

That they are a consequence of postmortem rigor in the heart muscle, as has been claimed by Sury, is denied by Geringer, who, however, admits that they may be agonal or pre-agonal. They occur more with death from slow bleeding than with death taking place quickly from the loss of a large amount of blood. As a rule, there was but little blood in the heart chambers. The subendocardial hemorrhages were not related in any way to arteriosclerosis or to age. In this report there is practically no reference to the influence that fibrillary contractions at the time of death or failure of all portions of the myocardium to cease contractions at the same time may have in causing these hemorrhages, although, as already stated, Geringer does suggest that they may be agonal.

E. R. LECOUNT.

ANEURYSMS OF THE CEREBRAL ARTERIES. K. SZEKELY, Beitr. z. gerichtl. Med. **8**:162, 1928.

Medicolegal postmortem examinations sometimes disclose extensive interleptomeningeal hemorrhage from ruptured aneurysms of the cerebral arteries as the explanation for sudden death. Because vomiting is often one of the symptoms of such hemorrhages, poisoning is suspected. In 11,500 necropsies at Vienna in the

Institute for Legal Medicine, there were 157 such deaths, or 1.4 per cent, and during the same period 348 from intracerebral apoplexy. A belief has long prevailed that the middle cerebral arteries are the seat of these aneurysms more than other arteries of the brain, but Szekely found the hemorrhages were far more frequently from aneurysms of the anterior communicating artery (table).

Another surprise in his summary is the greater number of such hemorrhages in women, the ratio of females to males being 65 : 35. In women, the aneurysms were mainly of the carotid system; in men, of the vertebral arteries and the vessels formed by them. Three fourths of all the 157 hemorrhages occurred in persons

Distribution of Aneurysms of Large Intracranial Arteries

	Per Cent		Per Cent
Anterior communicating	32.0	Left internal carotid	3.7
Right middle cerebral	15.0	Right internal carotid	2.5
Left middle cerebral	12.3	Left posterior cerebral	2.5
Basilar	9.0	Right communicating	1.5
Right anterior cerebral	6.2	Right vertebral	1.5
Left vertebral	5.7	Right posterior cerebral	0.6
Left anterior cerebral	5.5	Left communicating	0.6
		Inferior anteriol cerebellar	0.6

aged 35 or older. The persons were not all adults, four being 20 years of age or less, the youngest of whom was aged 8. The aneurysms were nearly all small, only four being hazelnut or cherry sized. They were commonly at the site of branching; eight where the vertebral arteries unite to form the basilar artery. Syphilis is said to have caused a small number, but many more were from atherosclerosis and high blood pressure. When death was not immediate, the symptoms, in addition to the vomiting referred to, were dizziness, headaches, etc., rather than focal symptoms.

E. R. LeCount.

Cataleptic Rigor Mortis Leading to Detection of Murder. W. H. Schultze, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:13, 1929.

The occurrence of rigor mortis immediately after death is rather rare, and only a few thoroughly investigated cases have been accepted. Some pathologists with an experience of many years have never observed this condition. The case reported by Schultze was that of a man shot through the head from right to left as he was sitting on a stool before a writing table. The body and the room and its other contents were taken in charge by the police at once, and when Schultze came he found the stool up-turned and the body prone with the right hand and arm underneath and the left arm bent, the thorax and abdomen not touching the floor. The back was a little bowed and the legs slightly bent. There was a pen in the right hand and a piece of paper in the left, both grasped firmly. It was concluded that rigor came on immediately when he was shot. It was present in all parts of the body.

The son-in-law at first denied having done the shooting, and was confounded when the body was turned over and writing materials found so tightly held; evidently he had not known that they were there. Subsequently, he confessed.

E. R. LeCount.

Help by the Mother During Labor, Causing Death of the Child. W. H. Schultze, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:21, 1929.

Difficulty in explaining the injuries and death of a newly born child finally resulted in tracing them to an arm presentation and self-delivery by the unwed mother when she was alone. The swollen left hand, the direction and location of

abrasions and nail scratches corroborated the story told by the mother. A fracture of the cranium and an injury of the brain occurred by pressure of the head in the birth canal, when the child was born "conduplicato corpore." The self-aid was successful because the woman was a multipara and labor several weeks too early; the weight of the child was only 1,700 Gm. The child was born alive and lived about two hours. For a time, the mother was suspected of infanticide.

E. R. Le Count.

Changes of Renal Blood Vessels in Experimental Poisoning with Bichloride of Mercury. B. A. Photakis and E. Nikolaides, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:28, 1929.

Swelling, shrinkage and necrosis of the endothelium of both the intertubular and glomerular capillaries, with hemorrhage and subsequent collapse and contraction of the malpighian bodies, were found in dogs given from 0.005 to 0.01 Gm. of bichloride of mercury per kilogram intravenously. The investigators maintain that the changes are not so exclusively in the epithelium as has been claimed, and that the term bichloride of mercury "nephrosis" is not suitable.

E. R. Le Count.

Proof of Arsenic Poisoning After Cremation. M. H. Remund, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:33, 1929.

Five years after the death of a man and the cremation of his body, suspicions of poisoning were aroused by attempts to blackmail the widow. From the ashes, which had been preserved in an urn, 47.2 mg. of arsenic was recovered. Of this, about 40 Gm. was accepted as belonging to the Paris green that the widow had used; the remainder was possibly derived from various other sources, such as the hardware of the casket, the urn, arsenic normally present in the body, etc. On this basis, it was concluded that the amount of arsenic in the body before cremation must have been at least 0.4 Gm. The lethal dose is from 0.1 to 0.2 Gm. Probably, a large factor in deciding as to the arsenic poisoning was the use of coke from the gas works in the crematory, such fuel being free from arsenic. Moreover, no other fuel had ever been used there.

Experiments were made with other bodies of persons dying from natural causes, one that of a person who had died from acute arsenic poisoning, and with bodies of lower animals. One interesting experiment was placing organs from an animal poisoned with arsenic under bones from a normal animal and finding that, by sublimation, the bones became heavily impregnated with arsenic. This is important because of its bearing on the common belief that large amounts of arsenic accumulate in the bones during life.

E. R. Le Count.

Suicide by Strangulation. A. M. Marx, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:104, 1929.

The opinion is generally held that it is impossible for a person to throttle or strangle himself so that death results. One reason for the holding of this view is that postmortem examinations alone fail to decide whether death is from murder or suicide. Marx reports two cases in which the circumstances left little doubt that self-strangulation had caused death. One concerned an insane mother who, after beating a daughter unconscious, strangled herself in bed by pulling a silk cord tight. The knot of the cord was on the left side of the neck, and the daughter was crippled from infancy and moved only in a wheel chair. In the second case, an insulated electric wire was wound about the neck nine or ten times, and where each coil lay against the loop at the end, the insulation was abraded because it had been pulled so tightly. The body was found in a farm field six weeks after the man had disappeared. A letter in his clothes stated his intention to commit suicide. His hand writing was identified; the content was a tale of domestic trouble.

E. R. Le Count.

IMPORTANCE OF THE DISTRIBUTION OF ARSENIC IN THE BODY. S. SCHÖNBERG, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:111, 1929.

Not only the amount of arsenic found in different parts of the body is important, but also its location is of value in determining how it was administered. Following a single, but not fatal, oral dose in rabbits, the rabbits being killed at different times, the arsenic disappeared from the content of the stomach and bowel first; reached its peak in the liver in the thirty-fourth hour; was absent from the kidneys but present in the brain in considerable amounts on the seventh day, and at this time was found in the skin and hair in $\frac{1}{18}$ of the amount given and in the liver in only $\frac{1}{100}$.

In two rabbits receiving a single dose intravenously and killed, one four and the other eighteen hours later, there were traces in the wall of the stomach and bowel, but none in their content. The distribution and amounts in those killed some time after the last of a number of doses were similar to what was found in those killed some time after a single dose. When killed earlier after the last one of a number of administrations, the quantity in the hide, as well as in the stomach, bowel and liver, definitely indicated multiple doses. Conditions in, and indications from, the bones were similar to those in and from the skin and hair. Arsenic is present in the brain for relatively long periods after a single dose.

E. R. LE COUNT.

SPECTROGRAPHIC EXAMINATION OF BULLETS. W. SCHWARZACHER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:226, 1929.

French investigators made a spectroscopic study of the composition of the projectiles of the "Big Bertha" used to bombard Paris during the World's War. In a few cases information gained by this method concerning the various metals of the missiles of revolvers and other small weapons has been of great use in prosecuting crime. Spectrum analysis of the metallic jackets of seven bullets of different calibers and of their lead cores, and also of four unmantled bullets, was made. Three of the naked bullets or cores contained copper and iron, or iron alone, in addition to lead; only one was pure lead. The mantles, however, had a much more varied composition, some containing as many as eight elements, including such rare metals as scandium and vanadium and zirconium. Cobalt, aluminium and silver were also found.

The weapons from some firms, although taking cartridges of different calibers, had mantles for the bullets with a similar group of metals in them. But this was not true for the bullets of other firms making guns of a particular kind. By dissolving the metals in nitric acid and using solutions of from 0.1 to 0.0001 per cent, some indication was obtained of the quantity of each metal present.

E. R. LE COUNT.

DEMONSTRATION OF FAT AND AIR EMBOLISM. OTTO SCHMIDT, Deutsche Ztschr. f. d. ges. ger. gerichtl. Med. **13**:231, 1929.

Two deaths are reported, one from fat embolism following a broken leg, the other from air embolism due to the use of a rubber syringe to bring on an abortion. In each body, the foramen ovale was patent, and the emboli were easily and promptly found grossly in the vessels of the choroid plexus. Since air readily enters veins torn across when the skull cap is removed, or both veins and arteries of the brain when such vessels are cut in removing the brain, the suggestion is made that possibly one or both procedures should be carried out under water.

E. R. LE COUNT.

DELAYED DEATH AFTER PENETRATING WOUNDS OF THE HEART. K. MEIXNER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:250, 1929.

Wounds penetrating the right auricle of the heart are not so abruptly fatal as are those penetrating other parts of the heart. But when the right auricle is torn

or perforated from in front, some of the large blood vessels are generally injured and death comes on quickly. There is a place between the mouths of the superior and inferior vena cava where the sinus of the right ventricle may be perforated and death may not occur so promptly. Meixner describes two such wounds, one, a laceration from blunt force, reported by Geringer (Beitr. z. gerichtl. Med. **3**:1, 1919); the other, a stab wound and his own observation. A third and much more remarkable case is that of a man who lived twenty-five days after he had shot himself, the bullet passing into the right ventricle, through the interventricular septum, through the base of the front mitral leaflet and into the wall of the left auricle, where it lodged. Infection of the wound set in and death was from pyemia and metastatic abscesses of the brain, rather than from wounds of the heart.

E. R. Le Count.

The Influence of Hydrocyanic Gas on the Color of Livores. W. Laves, Deutsche Ztschr. f. d. ges. gerichtl. Med. **13**:261, 1929.

Among the several theories for the bright red patches in the skin after death from prussic acid and its compounds, none is altogether satisfactory. The most acceptable assumes that they are a result of postmortem diffusion of the volatile acid in its gaseous state. Many experiments were made by Laves, all supporting such a view. The wide-spread belief that such pink or scarlet livores are a common effect of poisoning by HCN is not in accord with the facts. At the Institute of Legal Medicine in Graz during twenty years, twelve deaths, it was decided, resulted from HCN. One body had bright red patches in the skin with the blood a similar color. Two others had such red livores, but the blood was dark. No such changes were observed in the other bodies. Laves took pieces of skin containing the usual grayish-purple livores and after covering all but the external surface of some and the deeper surface of others with paraffin found they became bright red when exposed to HCN.

Other experiments were made with bodies of persons dead from natural causes and with conditions arranged to ascertain the effect of temperature and moisture on the rate of diffusion of HCN. It was learned that this gas diffuses about twice as fast as O_2, that some of the red color of cadaveric spots is due to O_2 entering the body from without and that diffusion of gases from without into the body is greatly aided by the moisture which collects on the skin when bodies are kept in cold, damp places. A diffusion of HCN outward toward the surface takes place when sodium cyanide, for example, is swallowed and when the gas kills persons who inhale it after entering buildings or the holds of ships too soon after its use as a vermifuge or a rat poison. The immediate death which it causes is also in agreement with postmortem formation of whatever color changes are made by this powerful swift poison.

E. R. Le Count.

New Interpretations from Postmortem Examinations. Raestrub, Deutsche Arch. f. d. ges. gerichlt. Med. **13**:291, 1929.

Of all the many varieties of objective evidence useful in medicolegal investigations that furnished by postmortem examinations is by far the most valuable. Raestrub believes that there is a marked and increasing tendency to overlook this, and that one of the many forces now responsible for belittling the significance of such evidence is incompetency of prosecuting officials and police engaged in criminal work. He deplores the accord between these officials and the effect that premature announcements resulting from their co-operation have on judicial proceedings. Another factor is the disposition to omit postmortem examinations and from such information as the police furnish and merely a cursory inspection of the outside of the body, to decide what caused death.

To illustrate his disapproval of this trend of affairs Raestrub, working under Kockel in the Institute of Legal Medicine, refers to several experiences which they had there at Leipzig. One was a plea of self defence made by a man who claimed

that the stab wound of the neck in his antagonist, who died, was incidental to a scuffle during which he simply tried to brandish the weapon and to use only the flat side of the blade. It was necessary to insist that a postmortem examination should be made. When this was made, a second deep stab wound into the thorax through the sternum and penetrating the aorta was found, a wound which must have been made with great force. Death was not, as had been claimed, from bleeding from the wound of the neck, but was internal and from the wounded aorta.

Another death thought to be from hemorrhage from radial blood vessels cut in the wrists was found by the postmortem examination to be from suffocation; the throat was plugged by a gag placed there by the woman when she tried unsuccessfully to kill herself; the important radial vessels were intact.

Two other murders were sex crimes. Both bodies were found in the same locality, one that of a girl, aged 13; the other, that of a boy of the same age. Thorough examination of the body of the girl, who was killed first, and of her clothes, left no other possible conclusion except that she was first assaulted and then drowned, and a report to that effect was given the authorities. A few days later, to the great astonishment of Raestrub and his colleagues, a statement appeared in the newspapers, that death from violence was improbable and that the injuries were from epileptic fits. This announcement came from an expert consulted by the police, and from one who, moreover, was not present at the examination of the body. Two years later, the boy was killed and again the newspapers opposed the conclusions of those at the Institute of Legal Medicine, who were forced to believe that both murders were probably committed by the same person.

E. R. Le Count.

Rupture of the Uterus. M. Krüger-Franke, München. med. Wchnschr. **75:** 132, 1928.

The mortality from rupture of the uterus is from 30 to 60 per cent. The author had six recoveries after hysterectomy in seven cases. He mentions as causes scars from operations involving the body of the uterus, abortion, puerperal fever, tears from labor, administration of preparations of hypophysis and defective training of physicians for obstetric work. Tears lengthwise in the cervix and tears in Bandl's contriction ring are the common locations of the lacerations. The patients are excited, have a redness of the face, suffer from repeated pains due to uterine contractions and have a tender lower uterine segment.

E. R. LeCount.

Technical

The Diagnostic Value of a Colorimeter for the Meltzer-Lyon Test. Edward Hollander, Am. J. M. Sc. **177:**377, 1929.

A colorimeter was used in 100 consecutive cases of cholelithiasis for the determination of the volume and color intensity of the dark bile obtained by duodenobiliary drainage, which is assumed to be derived from the gallbladder and which is commonly known as "B" bile. The bile was also examined microscopically. Gross disease of the gallbladder was present when the following observations were made: (1) no "B" bile; (2) from 1/5 to 2/5 of the normal amount of "B" bile without cholesterol crystals; (3) from 1/5 to 4/5 of the normal amount of "B" bile with agminated cholesterol crystals. When the amount of "B" bile was normal, the gallbladder at operation was practically normal in size and shape and was functioning through an open cystic duct. When agminated cholesterol crystals precipitated on particles of bile-stained débris were present in these cases, cholesterosis of the gallbladder with or without stones was found. In 4 per cent of the cases of cholelithiasis in which calculi were present in a normal-sized gallbladder, the amount of "B" bile was normal and agminated cholesterol crystals were absent.

Pearl M. Zeek.

THE VOLUME AND HEMOGLOBIN CONTENT OF THE RED BLOOD CORPUSCLE. MAXWELL M. WINTROBE, Am. J. M. Sc. **117**:513, 1929.

Simple methods are described for the calculation of the volume and hemoglobin content of the red blood corpuscle. Healthy young adult cells vary from 70 to 98 cubic microns in diameter and contain 28.7 by 10^{-12} Gm. of hemoglobin, the latter occupying about 33.3 per cent of the entire volume of the cell.

PEARL M. ZEEK.

SUGGESTIONS FOR STAINING TUMORS OF SPONGIOBLASTIC ORIGIN. NATHAN CHANDLER FOOT, Am. J. Path. **5**:215, 1929.

A method is described whereby the Globus-Hortega technic is combined with the hematoxylin-van Gieson stain, so that a polychrome picture results. It is believed that this gives slides that are more readily interpreted and have a greater similarity to routine sections than is the case with those impregnated with silver alone. They are also better suited to photomicrography.

AUTHOR'S SUMMARY.

METHODS FOR GROWING BRUCELLA ORGANISMS FROM FECES IN UNDULANT FEVER. H. L. AMOSS and MARY A. POSTON, J. A. M. A. **93**:170 (July 20) 1929.

About 1 Gm. of fresh feces was mixed in 50 cc. of sterile isotonic salt solution and shaken for a few minutes to insure thorough suspension. The suspension was filtered through four layers of number 1 hospital gauze to remove gross particles and centrifugated at half speed for three minutes to throw down other particles and larger bacteria. To the supernatant suspension, a sufficient amount of immune serum was added to make the total dilution 1 : 100, and after being shaken the mixture was placed in a water bath at 37 C. for two hours. The suspension was centrifugated at half speed for five minutes and the supernatant fluid discarded.

The precipitate was resuspended in isotonic salt solution, stirred and centrifugated at the same speed again. The supernatant fluid was again discarded and the procedure repeated twice. Finally the precipitate was spread with a bent glass rod on eosin-methylene blue plates, some of which were incubated at 37 C. aerobically and others in an anaerobic jar containing 10 per cent carbon dioxide.

Large clear colonies appeared after ninety-six hours. These were fished and the organism identified in the usual manner.

By this method a *Brucella* strain corresponding immunologically to Hygienic Laboratory strain 428 has been cultivated thirty-six times from the stools of a patient suffering from *Brucella* peritonitis and oophoritis. *Brucella abortus* (porcine) has been isolated from the stools of another patient in the sixteenth month of his infection.

BLOOD AND BONE-MARROW CELLS OF THE DOMESTIC FOWL. CLAUDE E. FORKNER, J. Exper. Med. **50**:121, 1929.

A simple, direct method of counting leukocytes of the fowl is described. Twenty-nine complete, morphologic studies of the blood of eleven domestic fowls are recorded. The characteristics of the cells found in the blood and bone-marrow are described in detail, and their relative numbers reported. The supravital technic, in which neutral red and Janus green are used, enables one to separate and classify accurately the confusing cells of the blood and bone-marrow. These studies provide a basis for future experimental studies on the blood and bone marrow cells of the fowl.

AUTHOR'S SUMMARY.

HISTO-TOPOCHEMIC EXAMINATION OF DISEASED ORGANS BY INCINERATION OF SECTIONS (SCHNITTVERASCHUNG). O. SCHULTZ-BRAUNS, Virchows Arch. f. path. Anat. **273**:1, 1929.

For years, attempts have been made in botany and in human histology to define chemical substances in the tissue itself. For technical reasons, the results have not been good. The usual methods of fixation alter most of the tissue substances, and most methods of staining have not the value of chemical reactions.

Frozen sections are made from unfixed material of any organ. The sections are kept at a uniform thickness of 15 mikrons for the purpose of comparison. Many tissues can be cut much thinner. Such thinner sections are treated as ordinary tissue slides for comparison and for exact localization. The frozen sections must be prevented from melting and from any contact with water. This is effected by keeping the microtome knife and the instruments with which the section comes into touch below zero. A second freezing chamber cools the microtome knife (from above). A frozen drop of water near the handle of the knife indicates the necessary low temperature. The sections are transferred to dry glass slides, which must be clean. The incineration is done in a quartz furnace through which moist oxygen passes. Such slides, when examined in reflected light show the ash substances exactly in their original location. In the beginning of the incineration, carbon forms; later on, at higher temperatures, the carbon is burned to carbon dioxide. During these two processes, minute details of the tissue structure become distinct because they have different resistance to the high temperatures (isotropic and anisotropic substance of muscle, nuclei of epithelial cells, etc.). Between 100 and 150 degrees, the connective tissue, especially when hyalinized, becomes opaque and seemingly doubly refractive; this is due to an increased amount of air in it. When the heating is too rapid, bubbles of tarlike substances may cause artefacts. The furnace contains a thermometer, and the heating is carefully regulated by an electric resistance.

In many instances, the amount and distribution of the ashes in the tissue are surprising. In blood vessels, much more of ashes is found than would correspond to the lime substances as stained with hematoxylin. The aorta of the new-born infant contains a little more of ashes than that of a one year old child. Increase of ashes and changes in their distribution are found in cartilage which looks normal in the usual slide. Fresh caseation contains little ash substance; old caseation much. Normal lung tissue of young persons is nearly ash-free, especially in tuberculous people. But the thin and normal-looking alveolar walls of a man aged 80 were full of ashes. It was the same with an atelectatic apical scar. Ash changes in epithelium are small. Some of the ashes are situated in living cells, but probably these cells are impaired in their metabolism (cartilage surrounded by inflammatory foci, inflamed gallbladder, heart muscle fibers near scars). The chemical differentiation of the ashes in the incinerated slide naturally is difficult, in view of the extremely small amounts of substances concerned. Brown color of ashes is due to iron contents; such ashes are mainly found in organs with stasis. For potassium, McCallum's method can be used. The hematoxylin reaction is no direct indicator for calcium; it is positive only in the presence of iron. The calcium salts can be recognized in an indirect way by their poor solubility. After a slide is washed in distilled water for ten minutes, only the calcium salts remain. Some of the ashes are bluish. This is due only to quantity; fine layers of ash substance appear blue no matter what they consist of. There are great new possibilities for this method.

ALFRED PLAUT.

Society Transactions

PHILADELPHIA PATHOLOGICAL SOCIETY

Annual Conversational Lecture, April 11, 1929

J. Harold Austin, *Presiding*

Some Contributions of Tissue Culture to Pathology. Warren H. Lewis.

The literature on the contributions of tissue culture to pathology is so extensive and varied that one can cover but a small part of it in a single lecture. There are already several hundred articles dealing with tumors, tuberculosis, viruses, immunity, cytotoxins, bacteria, phagocytosis, allergy and the effects of radium, x-rays and various chemicals and toxins on cells.

ALLERGY

The allergic reaction in tuberculosis had generally been regarded as the result of a reaction between antigen and antibody, but uncertainty existed as to whether it was cellular or humoral until Mrs. Lewis and Dr. Rich (1928) added to cultures of spleens and of washed white blood cells from normal and from allergic guinea-pigs, measured amounts of tuberculin. This showed in the clearest manner that the cells from allergic animals are more sensitive to the effects of tuberculin than are those from normal animals. They are killed by amounts of tuberculin in which normal cells grow freely. This sensitiveness of the washed cells of allergic animals was exhibited regardless of whether they were exposed to tuberculin in plasma of normal or of allergic animals. Tuberculin in allergic plasma had no more effect on normal cells than had tuberculin in normal plasma. They concluded that cellular injury and necrosis associated with allergy in tuberculosis result from a change in the individual fixed tissue and blood cells which renders them more sensitive to the products of the tubercle bacilli.

VIRUSES

The tissue culture method has been used rather extensively for the cultivation of viruses, beginning with the work of Lambert, Steinhardt and Poor in 1912 and Levaditi in 1913 on the virus of rabies. Levaditi cultivated the virus with ganglionic tissue from normal rabbits and by transferring it to fresh tissue every few days succeeded in keeping the virus potent for thirty days. He also succeeded in keeping the virus of poliomyelitis potent through many passages, in cultures of ganglions from an infected monkey.

More or less success has also been attained in the cultivation or preservation of the viruses of herpes, rabbit myxoma and the rickettsia of typhus fever and Rocky Mountain spotted fever for short periods with suitable tissues.

The most successful efforts have been those of Lambert and his co-workers (1913, 1914), Harde (1915), Gins (1916), Parker (1924), Parker and Nye (1925, 1929), Hach (1925), Cracium and Oppenheimer (1926), Carrel and Rivers (1927) and Haagan (1928) with the vaccine virus.

Lambert and his co-workers, Steinhardt and Israeli, laid small pieces of cornea of the normal rabbit in the virus for a few minutes and then explanted the pieces into plasma from a normal rabbit. Other cultures were prepared in the same way by using pieces of liver, heart and kidney. The vaccine virus increased in strength in cultres of the cornea, but died out in the cultures of the other tissues. The cultures of the cornea of an immune rabbit and the cultures of that of an inoculated normal rabbit were explanted into the plasma from an immune rabbit. Both the immune cornea and the immune plasma inactivated the virus. Lambert

and Harde found that the virus died when the tissue culture cells died and that the virus was inactive in those cultures in which for some reason the cells did not grow.

Parker (1924) used the virus-infected testes of the rabbit in normal plasma and added from every five to seven days fresh normal testis. The virus remained potent for fifty-four days through nine passages. He found Guarnieri's bodies in the tissues. Parker and Nye (1925) placed pieces of normal testis in vaccine from the testicle of the rabbit for five minutes and then made cultures in normal plasma, and by adding fresh rabbit testis every five to seven days they kept the virus potent; on the eleventh passage it was 51,000 times more potent than the original virus. After four months, however, the potency was lost.

Cracium and Oppenheimer cultivated the virus of calf vaccine for seventy-one days and found that the virus was bound up in some way with granules, the so-called Paschem bodies, which were obtained by centrifugating the lymph for from forty to sixty minutes. Carrel and Rivers were able to "step up" the potency of the virus by cultivating it with cornea or skin or brain of six to fourteen day chick embryos in a medium of chicken plasma and embryonic juice. More recently, Haagan (1928) succeeded in maintaining the virulence of the virus for eight months. He used infected testis of rabbit explanted into normal plasma with spleen extract as a growth-promoting substance.

The important points brought out by the work in tissue culture of viruses can be summed up as follows: 1. Viruses which become impotent in the course of a few hours in culture mediums at incubator temperature can be maintained and even increased in virulence during a period of several months when cultivated in the presence of living cells. 2. Viruses soon lose their virulence when the cells die. 3. Special types of cells, such as nervous tissue, corneal epithelium, skin and testis, are apparently essential for the cultivation of the special viruses. 4. The vaccine virus is apparently present in the plasma of infected animals and in or on the white blood cells of such animals. 5. There are no indications that the viruses exist in culture mediums at any great distance from the living cells.

CHICKEN SARCOMA

There are a number of points which seem to ally the Rous and other similar chicken sarcomas with the virus diseases. The cultivation of the Rous virus in the presence of living macrophages and the dying out of the virus in cultures when the cells die, the presence of the virus in the cell-free blood plasma of sarcomatous chickens and on washed white blood cells, and the transmissibility of the sarcomas by cell-free tumor filtrates and desiccated tumor powder would seem to ally them with the virus diseases and to separate them off from mammalian sarcomas. On the other hand, frequent metastases into the lungs, liver, spleen and heart would seem to indicate that they are related to malignant tumors.

The investigations of chicken sarcomas by means of tissue cultures have been concerned, for the most part, with the morphologic and cultural characteristics of the cells that migrate from the tumors and with the relation of the virus to these cells.

Carrel and Burrows (1910, 1911) obtained in cultures of Rous chicken sarcoma two types of cells, an inner zone of radiating spindle cells and of ameboid round cells, and an outer zone of ameboid round cells that had migrated farther and faster than the spindle cells. They were unable to find any morphologic characteristics differentiating them from normal cells, but tumors were produced when the cultures were inoculated into fowl. Since the spindle cells and ameboid cells were not very different in appearance and behavior from normal cells, it became important to obtain pure strains of the two types of cells and to determine by inoculation which type was malignant. Over ten years elapsed, however, before Carrel, in 1924, isolated in pure cultures the spindle cells or fibroblasts and the ameboid cells or macrophages by utilizing the differential action of serum and embryonic juice. The pure cultures of fibroblasts rarely produced tumors after the fourth passage, while pure cultures of the ameboid cells always produced

tumors when inoculated into chickens. From this Carrel concluded that the macrophage or monocyte was the "malignant" cell. Since the cell-free supernatant fluid from the cultures of the so-called "malignant" cells also produced tumors, and since it is obviously impossible to inoculate the macrophages without inoculating some of the medium, the malignancy of the cultures might equally well be ascribed to a virus that can survive and multiply in the presence of macrophages but not in the presence of fibroblasts.

Fischer (1924, 1926) also obtained pure cultures of chicken sarcoma cells (monocytes) and succeeded in carrying subcultures for over two and a half years without their losing malignancy.

The "malignant" cells according to Carrel are (1) short-lived, fragile and difficult to cultivate; (2) able to digest fibrin rapidly; (3) readily transformable into fibroblasts, and (4) able to reproduce the Rous principle.

Since Fischer succeeded in cultivating the cells for over two and a half years, they can hardly be considered as short-lived cells; nor can they be considered as fragile cells, for Fischer claims to have obtained from a single cell a culture that was carried on for several months. This proliferation of a single sarcoma cell is not due to any special proliferative ability, since, according to Fischer and Laser (1927), malignant tissues multiply less rapidly than normal ones in their cultures. The liquefaction of the plasma clot is one of the most striking differences between tumor tissue and normal tissue. The transformation of the sarcomatous monocytes or macrophages into fibroblasts occurs more frequently, according to Carrel and Fischer, than the transformation of normal monocytes into fibroblasts. Separate cultures of such transformed malignant macrophages are no longer able to reproduce the tumor, according to Fischer. The ability of sarcoma macrophages and of normal ones to bring about the reproduction of the Rous virus in vitro is the most interesting part of the work on chicken sarcoma. Rous and many others had previously shown that the virus loses its activity after suspension for twenty-four hours in a fluid medium. Carrel found that when the Rous filtered extract was added to flask cultures of normal embryonic pulp, of normal spleen or normal buffy coat growing in a medium of plasma, Tyrode solution and embryonic juice, the virus remained active and even increased. When fragments of normal buffy coat or of normal spleen were added from time to time and the cultures transferred every two or three weeks into new flasks, there was produced during a period of two months at least 1 cc. of highly virulent fluid every day. The quantity of active virus depends, according to Carrel, on the number of multiplying cells in the medium, and soon dies out if the cells die.

It has been claimed that normal monocytes and macrophages can be transformed into "malignant cells" by treatment with the following substances:

1. Filtered extracts of chicken sarcomas. Carrel considered that the addition of the Rous virus to cultures of normal macrophages from the spleen or buffy coat transforms the latter into malignant cells because the cultures produce tumors when inoculated into fowl. When the virus or filtered extract was added to the culture medium, free from cells, the virulence was lost after forty-eight hours at incubator temperature. These observations might equally well be explained on the assumption that the virus can survive or multiply in the presence of living monocytes or macrophages.

2. Arsenic. Fischer asserted that normal spleen from a seven day chick embryo cultivated in arsenic pentoxide produced, after a number of passages, malignant cultures that gave tumors when inoculated into fowl. Haagan also reported a somewhat similar transformation of normal monocyte cultures into malignant cultures after treatment with arsenious acid.

3. Tar. Laser reported the transformation of normal embryonic spleen cells into malignant sarcoma cells by cultivating them in plasma from a hen into which tar had previously been injected intravenously.

These astonishing reports on the transformation of normal cells into sarcoma cells by arsenic and by tar in vitro in a relatively short period of time are much in need of ample confirmation.

MAMMALIAN SARCOMAS

Cultures of mammalian sarcomas usually show in autoplasma or homoplasma two types of migrating cells: ameboid cells, (monocytes or macrophages) and spindle cells (multipolar cells or fibroblasts) (Carrel and Burrows, 1911, Jensen and Ehrlich, sarcomas and a fibrosarcoma in man; Lambert and Hanes, 1911, mouse and rat sarcomas; Lewis and Gey, 1923, Crocker mouse sarcoma no. 180; Policard, 1926, rat sarcoma; Lewis, 1927, Walker rat sarcoma no. 1; Fell and Andrews, 1927, Jensen rat sarcoma; Carrel, 1927, Crocker rat sarcoma no. 10; Carrel and Ebeling, 1928, Jensen rat sarcoma). The migration is somewhat similar to chicken sarcomas and consists of an outer zone of more rapidly moving macrophages and an inner zone of macrophages and spindle cells. The cultures produced, as a rule, more or less rapid liquefaction of the clot. Carrel (1927) and Carrel and Ebeling (1928) obtained pure cultures of fibroblasts from the Crocker rat sarcoma no. 10 by cultivating small fragments of the tumor in a mixture of chicken plasma, Tyrode solution and extract of embryonic chick. The macrophages disappeared after a few passages. When pure cultures of the fibroblasts were inoculated into rats small tumors appeared in from four to six days that developed slowly and finally killed the animal. These pure cultures of fibroblasts continued to proliferate for sixteen months and retained their malignancy during this period.

The spindle cells of the Crocker mouse sarcoma no. 180 differ somewhat from normal fibroblasts; they are larger, the cytoplasm is denser and more granular and the nucleus is large. The fibroblasts of the Walker rat sarcoma no. 1 (Lewis, 1927) show somewhat similar peculiarities and, in addition, according to M. R. Lewis and Lockwood, contain twice the normal number of chromosomes. The fibroblasts of Crocker rat sarcoma no. 10 are likewise generally larger and coarser than normal ones. According to Carrel, the malignant fibroblasts of the Crocker rat sarcoma no. 10 are similar to normal ones in their mode of locomotion, residual activity, duration of life in nonreplanted cultures and in their rate of growth. The colonies of the malignant fibroblasts are larger, as a rule, than those of normal ones, and the malignant fibroblasts liquefy rat plasma, while normal ones do not. They also turn phenol red golden yellow, while normal ones turn it a pinkish orange, indicating that sarcomatous fibroblasts produce more acid than normal ones. Both types of fibroblasts can multiply to an unlimited degree in chick embryonic juice. Calf liver digest will suffice for an unlimited proliferation of sarcoma, but not of normal fibroblasts.

Lambert and Hanes (1911) found that the Ehrlich rat sarcoma cells grew as vigorously in plasma from tumor-bearing animals and from normal animals as in the plasma from six types of immune rats. Similar results were obtained by Lewis (1927) with the Walker rat sarcoma. Tumor cells, like normal cells, often grow readily in plasma or in a mixture of plasmas of alien species. Lambert and Hanes (1911) and Lamber (1914) found that the plasma from guinea-pigs immunized with several subcutaneous injections of rat sarcoma was an extremely poor medium for the culture of rat sarcoma cells and normal fetal rat ectoderm as compared with normal guinea-pig plasma. Similar results were obtained with plasma of guinea-pigs immunized with injections of fetal rat ectoderm. Lumsden (1924, 1925, 1926, 1927) claimed that the plasma from different species of animals into which fragments of mouse or rat sarcoma had been injected becomes highly toxic to the cultures of the antigenic tumor, killing the cells within a few minutes. This toxicity is not entirely specific. Drew found that such serum has an injurious effect on normal tissues such as mouse kidney epithelium.

MAMMALIAN CARCINOMAS

The epithelial cell of carcinoma has long been considered as the malignant cell of this type of tumor, and its recognition in cultures was comparatively easy, since they migrate in sheets or tubules as do normal epithelia (Lambert and Hanes, 1911; Carrel and Burrows, 1911; Thomson and Thomson, 1914). Drew (1922, 1923), the first one to obtain pure cultures of mouse carcinoma epithelium,

made the interesting discovery that the addition of fibroblasts to pure cultures (pure epithelium plus pure connective tissue) caused the epithelium to form acini and that the whole culture strongly resembled a normal mouse mamma.

The carcinoma cells thus far cultivated apparently show no special morphologic characteristics that would differentiate them from normal epithelium. According to Fischer (1928), cultures of mouse carcinoma cells are more sensitive to changes in the hydrogen-ion concentration of medium than are cultures of normal cells and are far less resistant to changes on the acid side. The carcinoma cells suffer more from loss of oxygen than do normal ones, and Fischer thinks that the relatively high oxygen tension needed by carcinoma cells may be necessary for the decomposition and partial elimination of lactic acid produced in the cultures. Pure strains of epithelial cells from Ehrlich's mouse carcinoma and the Flexner-Jobling rat carcinoma have been cultivated for many weeks and have retained their malignancy. Carcinoma cells, like sarcoma cells, tend to liquefy the plasma clot rapidly.

The cytolytic effects of antiserums on actively migrating mouse cancer cells was tested by Kohn-Speyer.

The effect of x-rays on carcinoma and sarcoma cells in cultures has been studied by Kimura and on normal cells by Strangeways and Oakley and by Strangeways and Hopwood. In both instances, there was a definite diminution in the number of cells undergoing mitosis. The Gamma radiations utilized by Canti and Spear also had a definite effect in reducing the number of cells in mitosis.

Book Reviews

ARTHRITIS AND RHEUMATOID CONDITIONS, THEIR NATURE AND TREATMENT. By RALPH PEMBERTON, M.S., M.D., Physician to the Presbyterian Hospital, Philadelphia; Associate Professor of Medicine in the Graduate Medical School of the University of Pennsylvania. Price, $5. Pp. 354, with 42 engravings and 1 colored plate. Philadelphia: Lea & Febiger, 1929.

Pemberton's monograph is rather loosely knit and has decidedly too much detailed discussion of recent experimental work. Important as much of this material undoubtedly is, a more critical summation would have been desirable. Those who are interested in repeating or elaborating the experiments described must of necessity refer to the original papers, and to others there seems to be an unnecessarily large amount of description of methods. This criticism applies particularly to the section on dynamic pathology which deals with tissue and chemical alterations. To many who are interested in this subject it may also seem that the author's extremely brief summary of the bacteriologic investigations in arthritis, only to speak in considerable detail and at great length of the circulatory changes, sugar tolerance, blood count and other chemical and metabolic abnormalities, is not wholly justified by the present day conception of the arthritic problem.

Nevertheless, much valuable material is contained in this book. Wide clinical experience is manifest throughout. Differences in pathology and symptomatology are adequately discussed with a wholesome warning that the lines between cannot be too closely drawn. The description of the morbid processes in this work as elsewhere is based on the monograph of Nichols and Richardson published in 1909. The chapter on treatment covers 134 pages and is probably as adequate as possible. Surgical and orthopedic measures are touched on briefly as compared with their consideration in Fischer's new book on the same subject. For this there is ample justification considering the breadth of that subject alone.

On the whole, Pemberton's book is a satisfactory expression of the ever gaining attitude that arthritis is a many sided disease requiring continued and utmost endeavor in elucidation and in treatment.

PATHOLOGY FOR STUDENTS AND PRACTITIONERS. Authorized translation of the Lehrbuch der Pathologischen Anatomie by DR. EDWARD KAUFMANN, Professor of General Pathology and Pathological Anatomy and Director of the Pathological Department, University of Göttingen. Translated by STANLEY P. REIMANN, M.D., Pathologist and Director of the Research Institute of the Lankenau Hospital, Philadelphia; Assistant Professor of Experimental Pathology in the Graduate School of the University of Pennsylvania. Price, $30. Three volumes. Pp. LXII, 2452, with 1,072 illustrations. Philadelphia: P. Blakiston's Son & Company, 1929.

Dr. Reimann's translation of Kaufmann's "Lehrbuch" makes available to the English-speaking world one of the great textbooks and works of reference in pathology. Indeed it may be said that there is no other work that contains such a wealth of concise information in the field of pathologic anatomy. Written, as Kaufmann states in his introduction to the latest edition, primarily for the student of medicine, the author has handled with special care such subjects as are regarded the most important for those who are to be the future physicians. The treatment of the subjects has by no means been confined to pathologic anatomy, but, to complete the point of view, to make it clearer to the understanding and at the same time to maintain interest, excursions are made into embryology, anatomy, physiology and general pathology. The close connection between pathologic anatomy and practical medicine, between theory and practice is emphasized by numerous references to clinical data. How well the author has succeeded in

his task is shown by the ever-increasing popularity of this work in his own country as well as abroad. Dr. Reimann's translation faithfully follows the German text. The translation, however, has somewhat rearranged the divisions and subheadings, and the publishers have used a better type and better paper in the translation. Thus the work has been expanded from the original two into three volumes. For greater convenience, a complete index of sixty-two closely printed, double columned pages is included in each volume; the references to illustrations are in bold-faced type. While there is a complete system of cross-references to related subjects in the text, the index summarizes them for ready references. The exhaustive bibliography which takes up more than 200 pages of the third volume is a veritable treasure mine of information. There are references to the literature of nearly all civilized countries. In the majority of the citations, the full title of the paper quoted is given. In many instances, Kaufmann gives in a few words the essentials of the paper; thus a quotation is followed by the remark "Confirms Aschoff's views"; "The diagnosis of catarrhal icterus should be limited; primary injury to liver cells must be considered"; "The stomach had become invaginated into the duodenum, woman of 58, good picture." The scope of the work may be judged from the table of contents: I. Organs of Circulation, pp. 1 to 184; II. Blood and Lymph: Hematopoietic Organs, pp. 185 to 291; III. Respiratory Organs, 292 to 542; IV. Digestive Organs, 543 to 816; V. Osseous System, 817 to 1050; VI. Joints, 1051 to 1227; VII. Adrenal Glands, 1276 to 1291; VIII. Urinary Apparatus, 1292 to 1457; IX. Sex Organs, 1458 to 1803; X. Nervous System, 1804 to 2031; XI. Muscles, 2032 to 2060; XII. Tendon Sheaths and Bursae, 2061 to 2067; XIII. Skin, 2068 to 2224; Appendix (References to the Literature), 2225 to 2452; Index, I to LXII. The treatment of the various subjects is thorough, systematic and yet concise. The translator has done far more than render Kaufmann's "Lehrbuch" into readable English. He has enriched the work by more than 100 excellent illustrations, and throughout the books there are numerous additions, partly references to the newer American literature, partly personal views of the translator. The reviewer believes that the English-speaking medical world owes a debt of gratitude to Dr. Reimann for doing so well a laborious, but well worth while task.

THE SCIENTIFIC METHOD: ITS FUNCTION IN RESEARCH AND EDUCATION. TRUMAN LEE KELLEY, Professor of Education and Psychology, Stanford University. Price, $2.50. Pp. 195. Columbus, Ohio: The Ohio State University Press.

This little book contains five stimulating lectures on topics suggested by the title. The main subjects are the relation of method to the field of investigation, the function of the questionary, the units for measuring intelligence and achievement, the bearing of recent developments in science on problems of education and the mental traits of men of science. Pathologists will find the discussion of the fundamental principles of scientific research and the generalization of the mental traits of men of science especially interesting and valuable. The author has a fortunate gift of clear, vivid, often aphoristic statement. It is an admirable book that should be read by all who are concerned seriously with scientific research.

Books Received

TULAREMIA: HISTORY, PATHOLOGY, DIAGNOSIS AND TREATMENT. By Walter M. Simpson, M.D., Director of Diagnostic Laboratories, Miami Valley Hospital, Dayton, Ohio. Foreword by Edward Francis, Surgeon, U. S. Public Health Service. Price, $5. Pp. 162, with 53 illustrations and 2 colored plates. New York: Paul B. Hoeber, Inc., 1929.

GREEK MEDICINE. Being Extracts Illustrative of Medical Writers from Hippocrates to Galen. Translated and annotated by Arthur J. Brock, M.D. (Edinburgh), translator and commentator of Galen, etc. Price, $1.75. Pp. 256. New York: E. P. Dutton & Company.

THE FEMALE SEX HORMONE. By Robert T. Frank, M.D., Gynecologist to Mount Sinai Hospital, New York. Price, $5.50. Pp. 324, with 86 illustrations and 36 graphs. Springfield, Ill.: Charles C. Thomas, 1929.

PRAKTIKUM DER KLINISCHEN, CHEMISCHEN, MIKROSKOPISCHEN UND BAKTERIOLOGISCHEN UNTERSUCHUNGSMETHODEN. Ninth edition. By M. Klopstock and A. Kowarski. Price, 14 marks, bound. Pp. 524, with 51 illustrations and 25 colored plates. Berlin and Vienna: Urban & Schwarzenberg, 1929.

In this useful book, the practical methods employed in clinical chemistry, microscopy and bacteriology (including the commoner serologic tests) are described clearly and briefly. Histologic methods are not included. In this edition many parts have been revised and several recent methods introduced. The illustrations also have been improved and new ones added.

PETTIBONE'S TEXTBOOK OF PHYSIOLOGICAL CHEMISTRY, WITH EXPERIMENTS. Revised and rewritten by J. F. McClendon, Ph.D., Professor of Physiological Chemistry, Medical School, University of Minnesota, Minneapolis. Fourth edition. Price, $3.75. Pp. 368. St. Louis: C. V. Mosby Company, 1929.

THE BLOOD PICTURE AND ITS CLINICAL SIGNIFICANCE (INCLUDING TROPICAL DISEASES). A Guidebook on the Microscopy of Blood. By Prof. Dr. Victor Schilling, Physician-in-Chief, The First Medical University Clinic, Charité, Berlin. Translated and edited by R. B. H. Gradwohl, M.D., Director of the Pasteur Institute of St. Louis, and the Gradwohl School of Laboratory Technique, St. Louis. Seventh and eighth revised edition. Price, $10. Pp. 408, with 44 illustrations and 4 colored plates. St. Louis: C. V. Mosby Company, 1929.

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